

Diagnosis of Deep Venous Thrombosis by Radionuclide Tests

Carl-Magnus Edenbrandt



LUND 1986

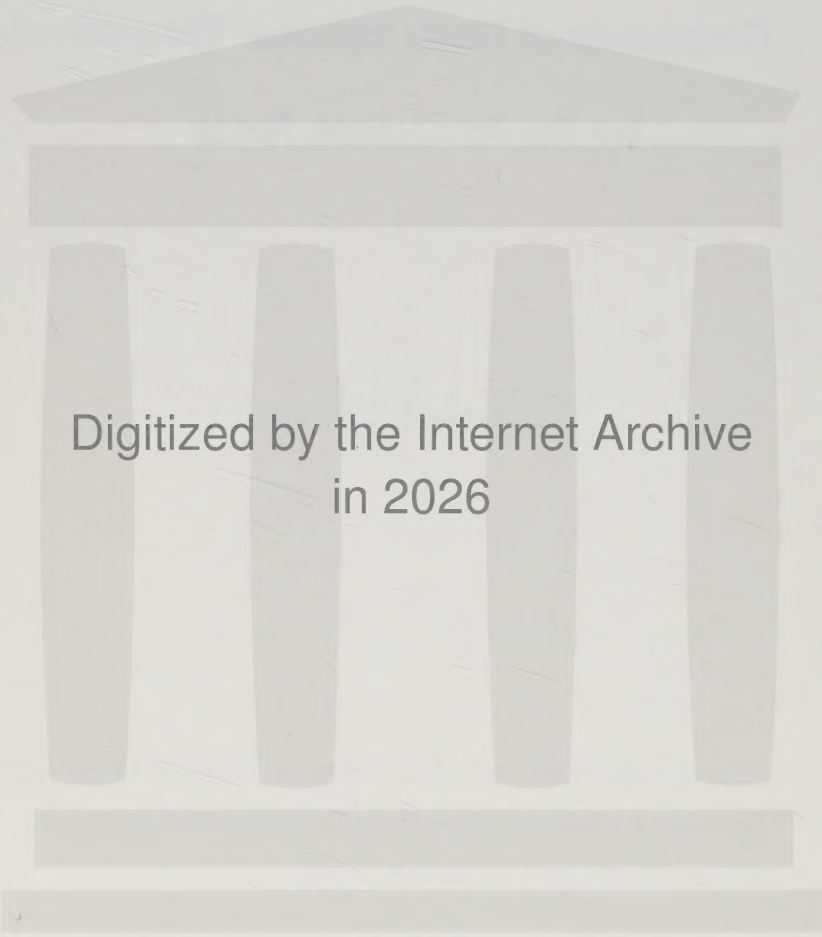
Diagnosis of Deep Venous Thrombosis

by Radionuclide Tests

Carl-Magnus Edenbrandt

From the Departments of Medicine and Clinical Physiology,
Lasarettet, Helsingborg and the Department of Medicine,
University of Lund, Lasarettet, Lund.

LUND 1986



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41552414_0008

This thesis is based on the following communications referred to in the text by their Roman numerals:

- I Edenbrandt C.M., Nilsson J. and Ohlin P. (1982) Diagnosis of deep venous thrombosis by phlebography and $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta Med Scand*, 211: 59-64
- II Edenbrandt C.M., Dahlström J.A., Nilsson J. and Ohlin P. (1984) Comparison between $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin and $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes in diagnosis of deep vein thrombosis. *Clinical Physiology*, 4 : 243-252
- III Edenbrandt C.M. and Brudin L. Criteria for detection of deep venous thrombosis by $^{99}\text{Tc}^{\text{m}}$ -plasmin test. Submitted to *Nucl Med Commun*
- IV Edenbrandt C.M., Hedner U., Nilsson J., Ohlin P. and Tengborn L. Return to normal of $^{99}\text{Tc}^{\text{m}}$ -plasmin test after deep venous thrombosis and its relation to vessel wall fibrinolysis. Submitted to *Eur J Nucl Med*
- V Edenbrandt C.M., Nilsson J. and Ohlin P. Follow-up of circulatory changes secondary to deep venous thrombosis with special regards to radionuclide tests. *Clinical Physiology*, in press.
- VI Ståhlberg F., Andersson L., Edenbrandt C.M. and Strand S.E. (1984) Quantitative in vivo and in vitro comparison between radiolabelled colloids, biomolecules and blood cells in their ability to diagnose deep venous thrombosis. *Nucl Med Commun* 5:741-762
- VII Edenbrandt C.M., Andersson L., Larsson I., Nilsson J., Ohlin P., Strand S.E. and Ståhlberg F. (1983) Diagnosis of deep venous thrombosis by $^{99}\text{Tc}^{\text{m}}$ - human serum albumin microcolloid. *Eur J Nucl Med* 8:332-334

Contents

Introduction	7
Present investigation	10
$^{99}\text{Tc}^{\text{m}}$ - porcine plasmin in diagnosis of established DVT	12
Mechanism of action for $^{99}\text{Tc}^{\text{m}}$ - plasmin in the diagnosis of DVT	15
Plasmin	15
Porcine plasmin	17
Labeling of porcine plasmin with $^{99}\text{Tc}^{\text{m}}$ - pertechnetate	18
Biokinetics and biodistribution of $^{99}\text{Tc}^{\text{m}}$ - porcine plasmin	21
Binding of $^{99}\text{Tc}^{\text{m}}$ - plasmin to the thrombus	23
Circulatory changes secondary to DVT	25
Comparison between $^{99}\text{Tc}^{\text{m}}$ - plasmin test and other radionuclide tests, particularly the ^{125}I - fibrinogen test	27
Interaction between radiopharmaceuticals and the thrombus	27
The ^{125}I - fibrinogen test, special remarks	29
Summary and conclusions	35
Acknowledgements	40
References	41
Appendix	
Articles	I - VII

Introduction

Deep venous thrombosis (DVT) is a common disorder in patients admitted to hospital care and most frequently localized to the legs (Havig,1977). A correct diagnosis is critical since anticoagulant therapy may prevent a fatal pulmonary embolism (PE) and a disabling post-phlebitic syndrome. On the other hand, one wishes not to expose patients without DVT to this potentially hazardous treatment.

It is widely accepted that a diagnosis of DVT based only on symptoms and signs is unreliable (Haeger, 1969; Hirsh & Hull, 1982). However, methods other than clinical diagnosis are still used infrequently in current practice (Prentice et al.,1982).

The most commonly used method today, is phlebography. It is considered the 'gold standard' to which all new methods are compared. However, phlebography is not suitable as a screening test, because it is painful and inconvenient to the patient, it carries a risk of post-phlebographic thrombosis, it involves a considerable effective dose-equivalent (VII) and it is not readily repeatable (Albrechtsson & Olsson, 1976; Lea Thomas & MacDonald,1978; Rabinov & Paulin,1983). Furthermore, it is expensive (Hull & Hirsh,1982a) and requires an experienced radiologist for interpretation.

The importance of diagnosing DVT in the legs and shortcomings of available diagnostic methods have encouraged development of radionuclide tests for diagnosis of DVT (Krohn & Knight,1977; Tjen,1981). There are basically two kinds of radionuclide tests. In radionuclide venography (RNV), the radiopharmaceutical is injected into a foot-vein and its transport by the venous blood as well as possible focal retention of the substance within the venous system are monitored by scintillation camera images. Areas of venous obstruction indicate DVT (Ryo et al.,1976; Hayt, Blatt & Freeman,1977;). At the present time, RNV does not offer

advantages over radiographic venography (Gomes, Webber & Buffkin, 1982) and will not be considered further in this discussion.

Most other radionuclide tests for diagnosis of DVT are based on the tracer principle (de Hevesy, 1964). It implies that a substance 'labeled' with a radioactive isotope should behave biologically, identically to a non-labeled substance. This concept was first applied to diagnosis of DVT by Hobbs & Davies (1960). They used the method of McFarlane (1958) to label rabbit fibrinogen with ^{131}I . The ^{131}I -fibrinogen was supposed to be converted to ^{131}I -fibrin by the thrombotic process and thus, to be incorporated into the forming thrombus after intravenous injection. These investigators found by external counting over the two hind legs that radioactivity was increased in a limb with venous thrombosis, but also when inflammation, wound healing, fractures or hematoma had been induced (Hobbs, 1962). Nanson et al. (Palko, Nanson and Fedoruk, 1964; Nanson et al., 1965) were the first to use ^{131}I -fibrinogen for diagnosis of DVT in man and Atkins & Hawkins (1965, 1968) introduced ^{125}I -fibrinogen. The ^{125}I has a lower photon energy allowing a light hand-held detector to be used and its long half-life (60 days) allowed for repeated measurements e.g. for follow-up of the incidence of DVT in the post-operative period. Flanc, Kakkar and Clarke (1968) and Negus et al. (1968) showed that increased radioactivity in one leg compared to the other or a continuous uptake of radioactivity over time in one leg, correlated well with DVT as shown by phlebography. Browse et al. (1971) modified the criteria for the ^{125}I -fibrinogen test to be more suitable for established DVT. Thereafter, the procedure for the ^{125}I -fibrinogen test has changed relatively little and the test has been used extensively for both diagnosis of established DVT and to follow patients at risk of developing DVT, e.g. after surgery (Browse, 1972; Kakkar, 1972; Hull & Hirsh, 1982b). However, a few drawbacks of the test have encouraged continuous research for a

superior radiopharmaceutical. The ^{125}I - fibrinogen test is rather non-specific and gives a high number of falsely positive tests. Furthermore, it takes up to 48 hours before a conclusive test result is obtained, and it carries a risk for transmitting diseases, e.g. hepatitis and AIDS. Therefore, other substances have been labeled with radionuclides to tailor a more suitable thrombus localizing agent (Tjen, 1981).

It was shown by Müllertz in 1956, that plasmin has a marked affinity for its substrate fibrin. This observation brought up the idea of labeling plasmin with ^{131}I and use this radiopharmaceutical to localize established DVT (Ouchi & Warren, 1962; Gomez et al., 1963). These authors showed an increased accumulation of radioactivity in the thrombotic leg of dogs. However, the labeling technique produced an unstable and chemically impure tracer substance. Alkjaersig, Fletcher and Sherry (1959) suggested that plasminogen activators are adsorbed to or diffuse into the preformed thrombus, thereby activating intrinsic clot plasminogen for subsequent thrombolysis. Based on this hypothesis the two plasminogen activators, streptokinase and urokinase, were labeled with ^{131}I and later $^{99}\text{Tc}^{\text{m}}$ (Siegel et al., 1972; Rhodes et al., 1972, Persson & Kempf, 1975). After initially encouraging reports, later results showed both non-specificity and unstable complex-formation of the radiopharmaceutical (Kempf, van der Linden and von Scheele, 1974; Weir et al., 1976; Olsson et al., 1979).

In an attempt to overcome the labeling difficulties, Persson and Darte (1977) used porcine plasmin, which could be labeled with $^{99}\text{Tc}^{\text{m}}$ at a low pH without denaturation of the protein and form a stable $^{99}\text{Tc}^{\text{m}}$ - plasmin complex in vitro. Shortly thereafter, Olsson et al. (Olsson et al., 1979; Nicolaidis & Olsson, 1982) published a preliminary report on $^{99}\text{Tc}^{\text{m}}$ - porcine plasmin for diagnosis of established DVT in man. The promising results of their study furnished the impetus for the present investigation.

Present investigation

The clinical value of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test was studied by comparison with phlebography in patients consecutively admitted to the medical emergency ward due to suspected DVT. Patients without DVT (negative phlebogram) were re-examined after 3-5 days in order to establish the most probable alternative diagnosis and to estimate the incidence of post-phlebographic thrombosis (I).

The significance of $^{99}\text{Tc}^{\text{m}}$ - plasmin uptake by the thrombus for the outcome of the plasmin test, was studied by comparing the distribution of $^{99}\text{Tc}^{\text{m}}$ - plasmin and $^{99}\text{Tc}^{\text{m}}$ - labeled erythrocytes in the same legs after intravenous injection (II).

An attempt was made to find new criteria that better discriminate between positive and negative test results (III).

Patients with a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test and DVT were followed to determine how soon the test returned to normal, to get information on when it could be used in patients with symptoms and signs suggestive of acute, recurrent DVT and with a history of recent DVT (IV,V).

To elucidate what determines the normalization time of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test, particular interest was paid to initial symptoms and signs, initial extension of DVT(according to phlebography), initial extension of positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test, duration of anticoagulant therapy and fibrinolytic activity in vessel wall and plasma (IV).

Patients with DVT were followed for six months with repeated $^{99}\text{Tc}^{\text{m}}$ -plasmin test, strain gauge plethysmography, distribution studies of $^{99}\text{Tc}^{\text{m}}$ -labeled erythrocytes and phlebography in order to characterize the morphological and functional changes accompanying DVT and their relation to the $^{99}\text{Tc}^{\text{m}}$ -plasmin test (V).

The thrombus uptake of injected $^{99}\text{Tc}^{\text{m}}$ -plasmin was studied in a rabbit model and compared to radiolabeled colloids, $^{99}\text{Tc}^{\text{m}}$ -fibrinogen, $^{99}\text{Tc}^{\text{m}}$ -human serum albumin, $^{99}\text{Tc}^{\text{m}}$ -erythrocytes, ^{111}In -labeled platelets, ^{111}In -labeled leukocytes, free and reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate (VI).

$^{99}\text{Tc}^{\text{m}}$ -human serum albumin microcolloid, a substance with no known relation to coagulation or fibrinolysis, was subsequently evaluated in consecutive patients suspected of having DVT (VII).

The following aspects of the work are discussed in this thesis:

$^{99}\text{Tc}^{\text{m}}$ -porcine plasmin in diagnosis of established DVT

Mechanism of action for $^{99}\text{Tc}^{\text{m}}$ -plasmin in the diagnosis of DVT

Comparison between $^{99}\text{Tc}^{\text{m}}$ -plasmin test and other radionuclide tests, particularly the ^{125}I -fibrinogen test.

$^{99}\text{Tc}^{\text{m}}$ -porcine plasmin in diagnosis of established DVT.

The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was evaluated in 105 patients, consecutively admitted to the medical emergency ward due to symptoms and signs in the legs suggestive of DVT (I). The sensitivity of the test was found to be 100 % and the specificity 51 %, when compared to phlebography. This result is well in accordance to others (Khan et al. 1981; Adolfsson et al.,1982; Olsson et al.,1982; Husted et al.,1984; Aronen et al.,1985) reporting sensitivities between 91 - 100 % and specificities between 33 - 63 %. However, Lückers et al. (1984) found a sensitivity of only 75 % and a specificity of 64 % in comparison with phlebography. The reason for this lower sensitivity is not clear, but it should be noted that in some patients the $^{99}\text{Tc}^{\text{m}}$ - plasmin test was not performed until 72 hours after admission and the patients were during this time on continuous heparin therapy.

Falsely positive $^{99}\text{Tc}^{\text{m}}$ -plasmin tests were noted mainly in patients with other inflammatory and/or vascular diseases in their legs, both in our study and by others (Adolfsson et al.,1982; Husted et al.,1984; Aronen et al.,1985). Khan et al (1981) found 3 patients out of 10 with positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test and negative phlebogram, to have clinical evidence of pulmonary embolism and a positive perfusion/ventilation lung scan. This finding indicates that the reference method, phlebography, does not always have a 100 % sensitivity for DVT. It has previously been pointed out by Browse et al. (1971) that also the sensitivity of the ^{125}I - fibrinogen test could be higher than phlebography.

We found no falsely negative $^{99}\text{Tc}^{\text{m}}$ -plasmin test in our limited number of patients but others (Adolfsson et al.,1982; Olsson et al.,1982; Husted et al.,1984; Lückers et al.,1984 and Aronen et al.,1985) have reported a total number of 14 patients, out of 338 examined, with negative test and still DVT according to phlebography. Most of these thrombi were

small and non-occlusive. One thrombus was found in a patient who had had symptoms for 2 - 3 weeks. Adolfsson et al. (1982) frequently performed bilateral phlebography and were thereby able to show that out of 5 patients with falsely negative $^{99}\text{Tc}^{\text{m}}$ -plasmin test, 4 had post-thrombotic changes or a superficial thrombophlebitis in the 'healthy' leg. Thus, patients without active DVT and only post-thrombotic findings in the 'healthy' leg, still showed an accumulation of radioactivity in this leg. Since the criteria for a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test involve a comparison between the count rates obtained of the two legs, the accumulation of radioactivity in the 'healthy' leg masked the accumulation of radioactivity in the other leg with DVT. Also the ability to detect bilateral DVT may be limited with these kinds of criteria, comparing the accumulation of radioactivity in the two legs. However, Olsson et al.(1982) were still able to detect two patients with bilateral DVT, because the suspected leg still showed an increased accumulation of radioactivity compared to the other leg.

The results for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test when compared to phlebography are similar to those for the ^{125}I -fibrinogen test compared to phlebography. A sensitivity of 71 % to 98 % and a specificity of 45 % to 85 % have been reported for the ^{125}I - fibrinogen test (Browse et al.,1971; Kakkar,1972; Mclvor et al.,1975; Olsson & Albrechtsson, 1980). However, the results of the ^{125}I -fibrinogen test were not obtained until 24 to 72 hours after injection of the radiopharmaceutical, while the $^{99}\text{Tc}^{\text{m}}$ -plasmin test results were obtained within 30 min. Falsely positive ^{125}I -fibrinogen tests were as frequent as for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test and found in patients with similar conditions. Falsely negative ^{125}I -fibrinogen tests were found in patients with small and non-occlusive thrombi and in patients with more than 7 days old thrombi.

An attempt was made to reduce the considerable number of falsely positive $^{99}\text{Tc}^{\text{m}}$ - plasmin tests by evaluating alternative criteria discriminating between positive and negative test results (III). The most efficient of our alternative criteria involved a comparison between the count rates obtained at three adjacent points of the suspected leg and the corresponding points of the other leg. Similar criteria have previously been proposed for the $^{99}\text{Tc}^{\text{m}}$ - plasmin test (Olsson et al., 1982) and also the ^{125}I - fibrinogen test (Browse et al., 1971). A limited increase in specificity of the test was achieved by performing only one measurement of leg points at 30 min after injection. Criteria that further increased the specificity simultaneously decreased the sensitivity. The most efficient criteria proposed in our study and the criterion previously proposed by Olsson et al. (1982) applied only at the 30 min measurement seem to be the most efficient possible for this radionuclide test.

We followed patients with DVT by repeating the $^{99}\text{Tc}^{\text{m}}$ -plasmin test and found a successive normalization of the test (IV). In patients with small and/or distal thrombi normalization took place within one to six weeks, but the test was still positive up to more than six months in patients with extensive or proximal DVT (IV). The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was normalized earlier than phlebographic findings at follow-up (V). These observations underline that a positive $^{99}\text{Tc}^{\text{m}}$ - plasmin test result should be evaluated with caution during the first 6 -12 months after a preceding DVT. To our knowledge no other report on follow-up with repeated radionuclide tests in patients with DVT has been published. Kakkar et al. (1969) made use of the longer half-life of ^{125}I (60 days) and showed that of 14 patients with a pathological ^{125}I - fibrinogen test lasting only 24 hours in the post-operative period, only one had DVT according to subsequently performed phlebography. This indicated rapid and spontaneous thrombolysis in 13 of the patients, according to the authors.

Bernstein et al.(1981) followed patients for one month post- operatively and showed that the ^{125}I - fibrinogen test was normalized more slowly in patients with extensive DVT than in patients with small DVT.

We have not experienced any adverse reactions after injection of $^{99}\text{Tc}^{\text{m}}$ - porcine plasmin in more than 500 patients, but the inherent possibility of allergic reactions must be kept in mind. We have also been reluctant to use the test in pregnant women.

Summarizing, the $^{99}\text{Tc}^{\text{m}}$ -plasmin test is non-invasive, easy to perform, rapid and convenient to the patient. Several studies have now shown a high sensitivity of the test using phlebography as a reference method, which makes it suitable as a screening-test. However, small thrombi, bilateral DVT and unilateral DVT combined with vascular diseases in the 'healthy' leg have been found in DVT patients with a negative $^{99}\text{Tc}^{\text{m}}$ -plasmin test. The test's low specificity necessitates an additional examination by phlebography in patients with a positive test. The overall results of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test are well in accordance with previously published results for the ^{125}I - fibrinogen test, but with the advantage of obtaining the result within 30 min and subjecting the patient to only half the effective dose-equivalent.

Mechanism of action for $^{99}\text{Tc}^{\text{m}}$ - plasmin in the diagnosis of DVT

Plasmin

Plasmin, a proteolytically active enzyme of the fibrinolytic system, is responsible for the degradation of fibrin and thus, thrombolysis (Collen, 1980). It is formed by activation of its precursor plasminogen. The concentration of native (Glu-) plasminogen in plasma is about $1.5\ \mu\text{M}$ and its plasma half-life about 2 days (Collen & Verstraete, 1975). Plasminogen is activated to plasmin by

intrinsic activators, involving at least factor XII, prekallikrein and high molecular weight kininogen (HMWK),

extrinsic activators, e.g. tissue plasminogen activator (t-PA) and urokinase,

exogenous activators, e.g. streptokinase.

The resulting two-chain plasmin molecule is composed of a heavy chain, containing five lysine- binding- sites (LBS) (Sottrup-Jensen et al.,1978) and a light chain, containing an active site similar to that of trypsin. The LBS are the molecular structures responsible for the high affinity of plasmin for fibrin.

Plasmin's broad enzymatic specificity is carefully regulated in vivo, presumably through its high affinity to its substrate fibrin and by an abundance of plasmin inhibitors (anti-plasmins). The main plasmin inhibitor of plasma is α_2 - antiplasmin (α_2 - AP), (Moroi & Aoki,1976; Wiman & Collen,1977). Its concentration in plasma is about 1 μ M and it has a half-life in plasma of about 2.5 days. α_2 - AP binds rapidly to free plasmin and forms a very stable 1:1 stoichiometric complex, thus protecting the breakdown of fibrinogen and other plasma proteins. The reaction rate between α_2 - AP and plasmin is one of the fastest described for protein-protein interactions and it has been calculated that the half-life of free plasmin in plasma is only about 100 ms. The half-life of the plasmin- α_2 - AP complex in plasma is reported to be about 0.2 days initially and about 0.5 days after equilibration between extra- and intra-vascular spaces (Collen & Wiman,1979). Plasmin molecules that are not free but actively degrading fibrin are presumably inactivated at a much slower rate since they have both their active site and the lysine- binding-sites protected from the inhibitor. Thus, infusion of free plasmin in vivo

or in vivo activation of native plasminogen will not cause free proteolytic activity in plasma and systemic fibrinogen breakdown, unless the antiplasmin activity is exhausted. Furthermore, plasmin found in excess of α_2 -AP reacts with α_2 -macroglobulin (α_2 -M), which has a plasma concentration of about 3.4 μ M and constitutes a second line inhibitor of free plasmin activity (Aoki & Harpel, 1984).

Porcine plasmin

In the present study, porcine plasmin (Lysofibrin™, NOVO A/S, Denmark) was used because it was readily available. This plasmin preparation is produced from purified porcine plasminogen, activated by addition of trypsin. After activation of plasminogen, trypsin is quantitatively removed and the plasmin is stabilized by addition of the aminoacid L-lysine. The porcine plasmin preparation was originally developed for use as a thrombolytic agent in patients with DVT. Thus, extensive investigations regarding its effect on coagulation factors, fibrinolytic activity, thrombolysis, toxicity and antigenicity have been performed (Storm, 1961; Storm, Larsen and Mogensen, 1961; Amris et al., 1963; Amris & Amris, 1963; Amris, 1966; Amris, Larsen and Brøckner, 1966; Larsen et al., 1966; Storm et al., 1974).

Hedner, Johansson and Nilsson (1978) reported effects on coagulation and fibrinolytic parameters after infusion of high dose (30 NOVO units = 10 mg/ kg body weight) porcine plasmin in patients with DVT. They found immediate decreases of both the rapidly reacting α_2 -AP and the α_2 -M levels after the infusion. A complex formation between pig plasmin and the α_2 -AP was demonstrated in crossed immunoelectrophoresis. These complexes were rapidly cleared from the circulation (< 18 hours). With the high dose given, free proteolytic activity in blood was demonstrated and

fibrinogen decreased in parallel with an increase of fibrin/fibrinogen degradation products.

Storm et al. (1974) reported on the thrombolytic effect of porcine plasmin . They gave an initial dose of about 10 mg plasmin/ kg body weight as an intravenous infusion over 1-1.5 hours, followed by 15 % of initial dose per hour for the following 5-7 hours. They found thrombolysis in 65 % of the patients in the plasmin treated group compared to only 15 % of the patients in the placebo group.

Possible differences in structure and activities of porcine plasmin compared to human plasmin have so far not been completely elucidated. However, we may conclude that porcine plasmin seems to act in man well in accordance with what is known about human plasmin. Small doses of free enzyme are rapidly inactivated by α_2 -AP and α_2 -M and subsequently removed from the circulation. Higher doses exert a proteolytic and thrombolytic effect in the circulation after the inhibitors are exhausted, as is also known for human plasmin. In our studies on the utility of porcine plasmin for diagnosis of DVT, however, we injected less than 1/400 of the amount given for therapeutic purposes. Thus, the risk for any side effects related to hemostasis may be considered negligible.

Labeling of porcine plasmin with $^{99}\text{Tc}^{\text{m}}$ -pertechnetate

In the present study (I), porcine plasmin was labeled with $^{99}\text{Tc}^{\text{m}}$ -pertechnetate according to Persson & Darte (1977). The labeling technique is based on a reduction of pertechnetate with stannous chloride at a pH of about 2. The radiochemical purity, i.e. the percentage of the radionuclide that is in the desired chemical form, was determined by gel chromatography column scanning (Darte & Persson, 1979). When using Sephadex G-25 medium (Pharmacia, Sweden), $^{99}\text{Tc}^{\text{m}}$ -labeled plasmin is

separated from three kinds of impurities, i.e. $^{99}\text{Tc}^{\text{m}}$ - complexes of lysine and chloride, free $^{99}\text{Tc}^{\text{m}}$ - pertechnetate and reduced, hydrolyzed $^{99}\text{Tc}^{\text{m}}$ (Persson and Darte, 1977). Using this method, the labeling efficiency was $91 \pm 2.5 \%$ (mean $\pm$ SD), lowest level 86 % (I). However, this method does not separate $^{99}\text{Tc}^{\text{m}}$ -plasmin from radiochemical 'impurities' of the same migration rate in the analytical system. Consequently, the necessity to select an appropriate gel for quality control measurements has been emphasized (Pettit et al., 1978). When we compared Sephadex G-25 fine and Sepharose 4B in the GCS system, we found lower radiochemical purities of radiolabeled colloids, fibrinogen and reduced pertechnetate in the Sepharose 4B system (VI). This latter gel has an improved resolving power for compounds in the molecular range of 10,000 - 500,000. Thus, today the 'true' labeling efficiency is difficult to assess and further development of test systems for control of radiochemical purity of radiopharmaceuticals is much needed.

Recently, Müller (1985) demonstrated lower labeling efficiencies for $^{99}\text{Tc}^{\text{m}}$ - labeled albumin preparations when the resolving capacity of the analytical system was improved. He used high pressure liquid chromatography (HPLC) to analyse the radiochemical purity. The radiochemical purity was found to vary from only 38 % to 75 % with HPLC compared to 43 % to 94 % with conventional column chromatography (Sephacryl S-300). Furthermore, he found that the labeling efficiency also varied inversely with the amount of tin colloid formed. When the molar tin/albumin ratio was $< 0.6:1$ the amount of $^{99}\text{Tc}^{\text{m}}$ - tin colloid was less than 10 % and the labeling efficiency 63 - 75 %. However, when the ratio of tin to albumin was 4.7:1 and 15:1, $^{99}\text{Tc}^{\text{m}}$ - tin colloid amounted to 30 - 40 % and the labeling efficiency was only about 40 %. In our $^{99}\text{Tc}^{\text{m}}$ - plasmin preparation, the molar tin/plasmin ratio was about 14 :1. Our analyses showed a high labeling yield although less than 100 %. This may

introduce concern as to what component in the injected preparation was actually measured in the patient. Furthermore, all the quality control tests were performed *in vitro* and consequently, some uncertainty about the radiochemical purity *in vivo* still remains.

It is, of course, important to make sure that the biological behavior of the labeled substance is not significantly altered by the labeling procedure. We analysed the porcine plasmin protein, before and after labeling, in gel chromatography (ACA 22 Ultrogel, LKB, Sweden and Sephacryl S-200, Pharmacia, Sweden) and found no differences in migration in the respective systems (unpublished). We also monitored the enzymatic activity of the protein with a chromogenic substrate (S-2251, Kabi, Sweden), before and after labeling, and found no significant difference (unpublished). The residual effect of $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin on serum left after formation of thrombi *in vitro* has been investigated (Tengborn & Hedner, 1981; Tengborn et al., 1982). With high doses of $^{99}\text{Tc}^{\text{m}}$ - plasmin both α_2 - AP and α_2 - M decreased. Crossed immuno-electrophoresis showed complex formation of $^{99}\text{Tc}^{\text{m}}$ -plasmin and α_2 -AP. Free proteolytic activity, as measured on fibrin plates, and fibrin/fibrinogen degradation products were not found unless the concentration of $^{99}\text{Tc}^{\text{m}}$ -plasmin was > 100 times what we maximally obtained in our studies (I, II). Both $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin and $^{99}\text{Tc}^{\text{m}}$ - human plasmin showed essentially the same results.

The importance of quality control is frequently omitted in publications regarding radiopharmaceuticals for diagnosis of DVT. Nevertheless, it may explain some of the differences found *in vivo* between identical substances that are labeled with different isotopes, or with the same isotope but with different labeling techniques. Furthermore, it is possible that radiochemical impurities may contribute substantially

to the accumulation of radioactivity seen in the diseased leg of patients with DVT after intravenous injection of radiopharmaceuticals (VI).

Biokinetics and biodistribution of $^{99}\text{Tc}^m$ -porcine plasmin

After an intravenous injection of $^{99}\text{Tc}^m$ -porcine plasmin, the radioactivity disappears rapidly from the blood. We sampled blood repeatedly during 60 min after the injection in six patients and found a clearance curve consisting of an initial, rapid component ($T_{1/2} \approx 5$ min) and a second slower component ($T_{1/2} \approx 180$ min). Only about 30 % of the injected activity remained in the blood at 30 min after the injection (unpublished). Khan et al. (1981) measured blood clearance by positioning the scintillation camera over the heart. They also found the clearance curve to consist of an initial rapid component and a second slower component. At least 50 % of the initial activity had disappeared from the circulation within 2 - 3 min after the injection. Persson et al. (1981) used scintillation camera measurements over the whole-body and found that about 30 % of the activity remained in the circulation after 30 min.

A major part of the from blood disappearing radioactivity is rapidly taken up by the liver and the spleen, with smaller quantities in the bladder and the kidneys (Khan et al., 1981). Persson et al. (1981) estimated that about 35 % of the injected activity was taken up by the liver and spleen within 30 min. These results are well in accordance with our own experience (unpublished). The initial liver uptake was in one study (Khan et al., 1981) found to be essentially the same as that for a $^{99}\text{Tc}^m$ -sulphur colloid preparation. Furthermore, the ratio of liver to spleen activity did not differ significantly between the $^{99}\text{Tc}^m$ -plasmin and $^{99}\text{Tc}^m$ -sulphur colloid preparations. The same authors also found marked similarities in the blood clearance curves of the two radiopharmaceuticals. They concluded that the $^{99}\text{Tc}^m$ -plasmin preparation 'is actively trapped by the

reticulo-endothelial cells of the liver and in this respect might well behave as a colloid' (Khan et al.,1981).

Turnover and biodistribution studies have also been performed with ^{131}I - labeled porcine plasmin (Amris et al.,1964). The disappearance of radioactivity from plasma showed a rapid phase ($T_{1/2} \approx 13$ min) and two slower phases with $T_{1/2} \approx 230$ min and $T_{1/2} \approx 0.8 - 2$ days, respectively. The radioactivity had equilibrated between the extra- and intravascular volumes after 4 - 6 hours and the distribution of ^{131}I -plasmin between these two compartments of the whole body was 5 - 4 : 1. The presence of ^{131}I - plasmin extravascularly was confirmed in additional dog experiments, in which the lymph was collected. Scanning over the liver and spleen, in patients, showed a steady decrease of activity from the initial measurement (after about 20 min) and during the following 2 hours. This is in contrast to the continuous and marked accumulation of radioactivity in the liver after intravenous injection of $^{99}\text{Tc}^{\text{m}}$ - plasmin.

We compared (II) the count rate of $^{99}\text{Tc}^{\text{m}}$ - plasmin with the count rate of $^{99}\text{Tc}^{\text{m}}$ -labeled erythrocytes, which were shown to remain intravascularly, both in blood samples and from external measurements over the legs. We found $^{99}\text{Tc}^{\text{m}}$ -plasmin to constitute about 1 % of the total counts within the blood and about 3 % of the total counts obtained over the legs, at 60 min after its injection. These data suggest that 2/3 of the radioactivity of the $^{99}\text{Tc}^{\text{m}}$ -plasmin preparation within the leg is distributed extravascularly. However, we also found the $^{99}\text{Tc}^{\text{m}}$ -plasmin/ $^{99}\text{Tc}^{\text{m}}$ - erythrocyte ratio to be particularly high over the ankle and knee regions. This may suggest an increased affinity to bone tissue, as is also known for some of the $^{99}\text{Tc}^{\text{m}}$ - colloids.

From the data presented above, it seems as if one part of the $^{99}\text{Tc}^{\text{m}}$ - plasmin preparation behaves as a 'colloid' and is rapidly taken up by the liver, spleen and bone tissue. Some of the $^{99}\text{Tc}^{\text{m}}$ -plasmin is rapidly

equilibrated between the intra- and extra-vascular water and exhibits a slower half life ($T_{1/2} \approx 3$ hours), not unreasonably off the half-life for the α_2 -AP-plasmin complex ($T_{1/2} \approx 5$ hours) (Collen & Wiman, 1979). Additional radioactivity is excreted by the kidneys and found in the urine. However, these data must be considered preliminary and further investigations with a longer follow-up (at least 6 hours) are much needed. One also wishes to know in which form the radioactivity exists in blood and extravascularly and if it changes during the observation period.

Binding of $^{99}\text{Tc}^m$ -plasmin to the thrombus

Tengborn & Hedner (1981) and Tengborn et al. (1982) have studied the binding of $^{99}\text{Tc}^m$ -plasmin to preformed thrombi, using the Chandler model for ex vivo thrombi (Chandler, 1958). Two ml of whole blood was introduced in a plastic loop and rotated until a thrombus was formed. Thereafter, $^{99}\text{Tc}^m$ -plasmin was added and the loop was rotated for another 5 min. The thrombus was then taken out, washed and examined by autoradiography. With the two highest concentrations of $^{99}\text{Tc}^m$ -plasmin, corresponding to 350×10^{-3} and 35×10^{-3} CTA units/ml, a definite lining of radioactivity around the thrombus was demonstrated. Using a lower concentration of $^{99}\text{Tc}^m$ - plasmin, corresponding to 3.5×10^{-3} CTA units/ml, no radioactivity was observed. The porcine plasmin was labeled with either 1950 MBq or 651 MBq $^{99}\text{Tc}^m$ - pertechnetate per 10 mg protein (about 130 CTA units) i.e. much in excess of the 300 MBq/ 10 mg plasmin in our preparations for the patient studies. Using $^{99}\text{Tc}^m$ - human plasmin gave essentially the same results. $^{99}\text{Tc}^m$ - human serum albumin gave a diffuse uptake of the whole thrombus, but not a definite lining. Free $^{99}\text{Tc}^m$ - pertechnetate gave no thrombus uptake at all. The presence of AMCA (tranexamic acid) made no difference, indicating that the binding

occured independently of intact lysine-binding-sites. The plasmin bound to the thrombus was proteolytically inactive and presumably complexed with α_2 -AP (Tengborn & Hedner, 1981). Summarizing their results, trace amounts of $^{99}\text{Tc}^{\text{m}}$ -labeled plasmin (human or porcine) do reach and are adsorbed to a preformed thrombus *ex vivo*, even in the presence of normal amounts of antiplasmins. However, the final concentration of plasmin in the loop had to be $35 - 350 \times 10^{-3}$ CTA units per ml in order to visualize the binding. 3.5×10^{-3} CTA units per ml gave no evidence of binding. The immediate concentration in blood of $^{99}\text{Tc}^{\text{m}}$ -plasmin given for diagnosis of DVT can be calculated to be about 8×10^{-3} CTA units/ml, i.e. in the lower range of detectability in the *in vitro* system. Since the *in vivo* system is considerably less sensitive, the results question the importance of the $^{99}\text{Tc}^{\text{m}}$ -plasmin taken up by the thrombus for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test. Furthermore, only 4.3 - 9.6 % of the total radioactivity added to the loop was found to be adsorbed to the thrombus (Tengborn, personal communication).

We examined the thrombus uptake of $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin in a rabbit model (VI). The thrombus-to-background ratio *in vivo* and thrombus-to-blood ratio *in vitro* were only slightly more than 1, and not significantly different from the ratios obtained for free $^{99}\text{Tc}^{\text{m}}$ -pertechnetate and $^{99}\text{Tc}^{\text{m}}$ -human serum albumin, after injection of the radiopharmaceutical far away from the thrombus site.

We also compared the distribution of $^{99}\text{Tc}^{\text{m}}$ -plasmin with $^{99}\text{Tc}^{\text{m}}$ -labeled erythrocytes, along and between the legs by measuring over the leg points of patients suspected of having DVT (II). We found no significant difference in distribution of the two radiopharmaceuticals between the legs in either patients with DVT or patients without DVT. Our results suggested that the amount of $^{99}\text{Tc}^{\text{m}}$ -plasmin bound to the

thrombus was negligible.

Dahlborn et al. (1984) and Lückers et al. (1984) have used scintillation camera images in an attempt to localize the accumulation of radioactivity in the legs after an intravenous injection of $^{99}\text{Tc}^{\text{m}}$ -plasmin. 'Hot spots' were never found in the 36 patients with DVT, each injected with about 80 - 100 MBq of $^{99}\text{Tc}^{\text{m}}$ -plasmin. Rather a diffuse increase of radioactivity was found in the diseased leg, more suggestive of blood pooling. In addition, one patient with DVT was examined with emission computerized tomography (ECT), but the 'blurred image' was interpreted by the authors as 'indicating increased blood pooling rather than a string-shaped uptake, which suggested that the main isotope activity was not in the thrombus' (Dahlborn et al., 1984).

In conclusion, trace amounts of injected $^{99}\text{Tc}^{\text{m}}$ - plasmin are presumably bound to the thrombus. However, the major part of the injected radioactivity is not found in the thrombus but in the blood and extravascularly at about 60 min after the injection. Thus, other mechanisms than a specific thrombus uptake seem to account for the increased count rate found over the leg with DVT compared to the other leg, in the $^{99}\text{Tc}^{\text{m}}$ - plasmin test.

Circulatory changes secondary to DVT

Comparing the distribution of $^{99}\text{Tc}^{\text{m}}$ - erythrocytes between the two legs, we found that both distal and proximal DVT induced markedly increased count rates over the diseased leg (II, V). This suggests either an increased pooling of blood in the thrombotic leg or a more superficial localization of the blood flow, i.e. a decreased blood-detector-distance. A higher count rate over the diseased leg compared to the other leg after intravenous injection of $^{99}\text{Tc}^{\text{m}}$ - labeled erythrocytes has also been observed by others (Beswick et al., 1979; Kempf & van der Linden, 1981). When the

procedure was repeated six months later, we found no major difference in count rate between the two legs, indicating a normalization of blood flow in the thrombotic leg (V).

The hemodynamic changes secondary to DVT were also studied by strain gauge plethysmography (V). Four out of five patients with proximal DVT had decreased calf volume expansion (CVE) in response to an induced congestive pressure. In this situation, it has been suggested that the veins in the calf are filled with thrombi, expanded by the increased i.v. pressure distal to the thrombus or compressed by an increased extravascular pressure, e.g. edema, impairing their ability to expand in volume in response to the proximal stasis (Sumner,1982). All patients with proximal DVT had a decreased maximum venous outflow (MVO) after the congestive pressure was released. Thus, the outflow of blood from the leg was compromised, presumably due to the obstructing thrombus (Sumner,1982). When repeated after six weeks and six months, respectively, we found a successive normalization of plethysmographic results indicating improved venous outflow from the leg. All our plethysmographic findings are well in accordance with other investigations (Dahn & Eiriksson, 1968; Hallböök & Göthlin, 1971; Ahlbäck, Bygdeman and Waltz, 1977; Jay et al., 1984).

Both symptoms and signs and the $^{99}\text{Tc}^{\text{m}}$ -plasmin test results seemed to normalize in parallel to results of the $^{99}\text{Tc}^{\text{m}}$ - erythrocyte test and plethysmography (V). However, repeated phlebography after six months still showed marked morphological changes in spite of the improved non-invasive tests. The phlebographic findings are well in accordance with previous reports (Bergvall & Hjelmstedt, 1968; Young, Thomas and Browse, 1974; May & Nissl, 1979; Rabinov & Paulin, 1983).

The similar behavior of the three non-invasive tests, both in the acute phase and during six months follow-up, suggests that a positive $^{99}\text{Tc}^{\text{m}}$ - plasmin test primarily reflects hemodynamic changes secondary

to the DVT rather than a specific binding of the radiopharmaceutical to the thrombus. However, the $^{99}\text{Tc}^{\text{m}}$ - plasmin test does not simply monitor changes in blood volume and blood flow of the diseased leg since the radiopharmaceutical is distributed between intra- and extravascular volumes. Extravascular changes, e.g. edema, or lymph flow must influence the amount of the $^{99}\text{Tc}^{\text{m}}$ - plasmin preparation in the extravascular compartment. An obstructing thrombus is known to increase the venous pressure distal to its origin, which promotes edema formation, increased lymph flow and redirection of the blood flow to collateral vessels (Strandness & Sumner, 1975). Thus, the marked intra- and extra-vascular changes accompanying DVT all seem to influence the outcome of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test. This may also be the mechanism of action for other radiopharmaceuticals, even those with a presumed thrombus affinity.

Comparison between $^{99}\text{Tc}^{\text{m}}$ - plasmin test and other radionuclide tests, particularly the ^{125}I - fibrinogen test

Interaction between radiopharmaceuticals and the thrombus

Using a rabbit model (VI), some radiocolloids (antimony colloid, tin-sulphur colloid, sulphur colloid, albumin microcolloid), plasmin, fibrinogen, albumin and blood cells, as well as free and reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate were all shown to be characterized by low thrombus uptake, when injected into a peripheral vein for transport by the general circulation to the thrombus. No compound showed a thrombus-to-background ratio of more than 2.2 : 1 and the thrombus-to-blood activity ratios were about 5 : 1, at about 30 min after injection. Furthermore, no compound showed more than 1 % of injected activity per gram of thrombus. Vieras et al. (1980) found a thrombus-to-blood (T/B) activity ratio of 3.3 : 1 for $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in dogs. Bardfeld, Rand and Goldsmith (1981) found T/B ratios of 3 - 11 : 1 for $^{99}\text{Tc}^{\text{m}}$ - sulphur colloid in rats. Siegel et

al. (1972) studied ^{131}I - streptokinase in dogs and reported a T/B ratio of 8 : 1, while Rhodes et al. (1972) reported a T/B ratio of 8.2 : 1 for $^{99}\text{Tc}^{\text{m}}$ - urokinase. Harwig et al (1976) reported T/B ratios of 10.5 - 13.9 : 1 for $^{99}\text{Tc}^{\text{m}}$ - fibrinogen. Thus, most other studies have found more radioactivity in the thrombus than in blood but the T/B ratio is generally low (< 10) and so, this finding is not limited to $^{99}\text{Tc}^{\text{m}}$ - plasmin.

Whether the behavior of the studied radiopharmaceuticals in the animal model (VI) was valid also for man, was subsequently examined. We chose human serum albumin microcolloid (HSAC), which has no known relation to coagulation or thrombi and is easily available for use in patients (VII). The study was organized identically to the $^{99}\text{Tc}^{\text{m}}$ -plasmin study, except that the radiopharmaceutical was changed. Using $^{99}\text{Tc}^{\text{m}}$ - HSAC gave similar sensitivity and specificity as attained with the $^{99}\text{Tc}^{\text{m}}$ - plasmin test. The result showed that this substance, with no relation to hemostasis and a low thrombus uptake in the animal model, still was useful for diagnosis of DVT in patients.

Bardfeld et al.(1983) reported on $^{99}\text{Tc}^{\text{m}}$ - sulphur colloid for diagnosis of DVT in 25 patients. They found a sensitivity of 79 % and a specificity of 91 % compared to phlebography. Scintillation camera images of the legs showed a diffusely increased activity in the diseased leg rather than a focal uptake. Kemp, van der Linden and von Scheele (1976) used $^{99}\text{Tc}^{\text{m}}$ - tripolyphosphate for diagnosis of DVT in 38 patients. They compared the uptake of the radiopharmaceutical in the suspected leg with the other leg and a ratio of more than 1.15 :1 was considered pathological. Sensitivity was found to be 94 % and specificity 65 % compared to phlebography. Kemp & von Scheele (1976) compared free sodium $^{99}\text{Tc}^{\text{m}}$ - pertechnetate and phlebography for diagnosis of DVT in 20 patients. An activity ratio of >1.15 :1 between the two legs indicated thrombosis. Sensitivity was 77 % and specificity 86 %. The results suggested that also

a substance not involved in hemostasis tends to localize in the area of thrombosed veins. Dilated vessels and passive congestion seemed to be factors promoting increased count rate in the thrombotic leg, rather than a specific thrombus uptake.

The ^{125}I -fibrinogen test, special remarks

In our studies of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test in patients with DVT, we found a difference in count rate between the normal and diseased leg already 30 min after the injection, although the specific uptake of $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin by the thrombus was found to be negligible (I, II, VI). On the other hand, the ^{125}I -fibrinogen test is reported to become positive only 24 - 72 hours after injection of the radiopharmaceutical, in spite of the fact that ^{125}I -fibrinogen is supposed to be actively incorporated into the thrombus. This would appear to be in conflict with our findings. However, this conflict might be due to the criteria used to identify a positive test.

The ^{125}I -fibrinogen test is performed in the following way (Browse, 1972; Kakkar 1972): The patient is in the supine position with the legs elevated 45° to decrease blood pooling. One hundred μCi (= 3.7 MBq) ^{125}I -labeled human fibrinogen (about 0.5 - 1 mg) is injected into an armvein. One hour later, the radioactivity is measured over the heart and over points distributed from the foot to the inguinal ligament, with a hand-held scintillation detector. The measurements are repeated at 24, 48 and sometimes 72 hours after the injection. The count rate measured at different points along each leg is expressed as a percentage of the precordial count rate and is referred to as the 'percentage heart uptake' for each point. A difference in percentage heart uptake of 15 % or more between adjacent points of the same leg or between corresponding points of the two legs, which persists and continues to rise from one

measurement to another is considered indicative of DVT (Negus et al., 1968). Browse et al.(1971) found that if there were three adjacent points each greater by 5 % than the same three points on the other leg, expressed in percentage heart uptake ($3 \times 5 \%$), this criteria was as accurate as the 15 % criteria, for established DVT.

Olsson and Albrechtsson (1980) used criteria modified after Browse et al. (1971), ($3 \times 4 \%$, percent heart uptake) in consecutive patients with symptoms and signs suggestive of DVT. They found that 62 % of the thrombi gave a positive ^{125}I -fibrinogen test already after one hour, 71 % after one day and 98 % after two days. At the same time specificity decreased from 72 % at one hour to 52 % after two days. Thus, similar sensitivity and specificity (98 % and 52 %, respectively) were achieved with the ^{125}I - fibrinogen test after two days as were obtained within 30 min with the $^{99}\text{Tc}^m$ - plasmin test. The criteria for a positive $^{99}\text{Tc}^m$ - *plasmin test* according to Olsson et al. (1982) are derived using the following formula:

$$Q = \frac{\text{CR}}{\text{CR} + \text{CL}} \times 100 - 50$$

where CR = count rate at a point of the right leg and

CL = count rate at the corresponding point of the left leg

A positive test is defined as $Q \geq 3$ (or $Q \leq -3$) in each of three adjacent pairs of measuring points of the two legs, or if $Q \geq 13$ (or $Q \leq -13$) for one pair of corresponding points, provided the suspected leg shows the highest count rate. These criteria imply a direct comparison of the count rates obtained at the right and left leg.

In the ^{125}I -fibrinogen test the count rate at a point of the right leg (CR) is expressed as a percentage of the count rate over the heart (CH), as is the count rate at a point of the left leg (CL). The criteria for a positive ^{125}I -fibrinogen test is 'a difference in percentage uptake of 15 % or 3 x 5 %, or more between corresponding points of the two legs' (Browse et al., 1971), i.e.

$$K = \frac{\text{CR}}{\text{CH}} \times 100 - \frac{\text{CL}}{\text{CH}} \times 100$$

where $|K| \geq 5$ in three adjacent pairs of leg points, or $|K| \geq 15$ in one pair of corresponding leg points, respectively. This formula can easily be transformed to:

$$K = \frac{\text{CR} - \text{CL}}{\text{CH}} \times 100$$

and thus, it is easy to understand that these criteria are not only dependent upon a direct comparison between the counts of the right and the left leg, but also dependent upon the counts over the heart. If the count rate over the heart (CH), i.e. in blood, is high (i.e. immediately after injection) it will be difficult for a certain difference (CR - CL) to reach the critical $|K| \geq 5$, or 15, level for a positive test. However, when CH is low ($T_{1/2} \approx 3-4$ days for fibrinogen; McFarlane, 1963) the same difference may now become significant. In the same way, if CR and CL both increase in absolute counts but their relation CR/CL remains the same, it will be easier to reach a significant $|K| \geq 5$ or 15. A quotient CR/CL of 1.13 :1 is significant for the plasmin test ($Q = 3.05$) and independent of the absolute count rate.

$$Q = \frac{1.13}{1.13 + 1} \times 100 - 50$$

For the ^{125}I - fibrinogen test, a quotient CR/CL of 1.13 :1 is not significant for low count rates of CR and CL but significant for high count rates of CR and CL, in relation to CH.

Example: If CR/CL = 1.13

- a) CR = 1,130 counts/ time
CL = 1,000 counts/ time
CH = 10,000 counts/ time

K = 1.3 (negative ^{125}I - fibrinogen test)

- b) CR = 4,520 counts/ time
CL = 4,000 counts/ time
CH = 10,000 counts/ time

K = 5.2 (positive ^{125}I - fibrinogen test)

Thus, the criteria used for the ^{125}I - fibrinogen test are not only dependent upon the relation of CR to CL but also upon the relation of CR, and CL, to CH. The count rates of CR and CL will decrease more slowly with time than CH, since ^{125}I - fibrinogen is gradually distributed to the extravascular space. After 2-3 days, 20-30 % of the ^{125}I - fibrinogen is extravascular and 70-80 % intravascular (McFarlane,1963). Even though the relation between the two count rates, CR and CL, may still be the

same, the difference (expressed in percentage heart uptake) may now be significant. Thus, if one uses the established criteria for the ^{125}I -fibrinogen test instead of a direct measurement of the relation between CR and CL, as is done in the plasmin test, one either has to wait until CH has decreased significantly ($T_{1/2} \approx 3 - 4$ days; McFarlane, 1963) or until CR and CL both have increased in relation to CH. At this later time the ^{125}I -fibrinogen test may be positive, even though the relation between CR and CL is the same, as measured by the $^{99}\text{Tc}^{\text{m}}$ -plasmin test. This explains the increased sensitivity and decreased specificity of the test with time, as was found by Olsson et al. (1980). The foregoing argumentation is independent of whether or not ^{125}I -fibrinogen is taken up by the thrombus.

The reason for expressing the count rates obtained from the legs as percentages of the count rate over the heart was the objective of doing repeated measurements over several days in post-operative patients. Hereby, Negus et al. (1968) wanted to detect thrombi forming during any of the days in the post-operative period. Nanson et al. (1965) and Atkins and Hawkins (1965) had expressed the measurements over the legs in counts per min but as the count rate decreased with time, due to the metabolism of ^{125}I -fibrinogen, it was difficult to make a comparison between serial measurements over the same point on the limb. By expressing the count rate as a 'percentage of the heart count rate', Negus et al. (1968) wanted to correct for the biological decay of the labeled fibrinogen and thus, make the comparison easier. However, the utility of the ^{125}I -fibrinogen test for diagnosis of established DVT may have been hampered by these less advantageous criteria.

There is general agreement in the field that an established DVT incorporates ^{125}I -fibrinogen very slowly (Browse, 1972; Krohn and Knight, 1977). It is also well known that less than 2 % of the injected

^{125}I -fibrinogen is extravascular at 15 min after injection (Amris & Amris, 1964). We may therefore expect similar results for ^{125}I -fibrinogen, 5 min after injection as we obtained for $^{99}\text{Tc}^{\text{m}}$ -labeled erythrocytes (II). The latter results did not significantly differ from the results of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test. Thus, we may conclude that by changing criteria for the ^{125}I -fibrinogen test from the criteria proposed by Negus et al. (1968), Browse et al. (1971) and Olsson and Albrechtsson (1980) to the criteria used for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test (Olsson et al., 1982) we may obtain similar results with the two radiopharmaceuticals.

The thrombus uptake of radiolabeled fibrinogen compared to remaining activity in the blood has been studied by only a few investigators. We found the thrombus-to-blood ratio of specific activities (T/B) to be about 5 :1 for forming thrombi and about 2 :1 for preformed thrombi in the rabbit model (VI). Harwig et al. (1976) found a T/B ratio of about 10 :1 one to three days after injection of $^{99}\text{Tc}^{\text{m}}$ -fibrinogen or ^{125}I -fibrinogen in dogs with preformed thrombi. Browse et al. (1971) found a T/B ratio of 4 :1 in a patient who died 48 hours after being given ^{125}I -fibrinogen and who was subjected to autopsy. Thus, trace amounts of radiolabeled fibrinogen is incorporated into preformed thrombi both in animals and man. However, as can be calculated and has been shown by Bernstein et al. (1979), less than 4 % of the radioactivity originating from a thrombus located at a depth of 4 cm from the skin surface can be detected with commonly used detectors. This is due to energy losses of the emitted photons through tissue attenuation and thrombus - detector distance. Furthermore, both Browse et al. (1971) and Olsson and Albrechtsson (1980) observed that even small thrombi were as easily detected with a 'three-point-criteria', covering a wider surface area, as a more focused 'one-point-criteria'. These findings all throw some doubt

upon the possibility to detect the radiolabeled fibrinogen in the thrombus. Thus, it is apparently not settled if the thrombus uptake of ^{125}I -fibrinogen is of significance for the ^{125}I -fibrinogen test.

It has been reported by Hull and Hirsh (1982b) that the ^{125}I -fibrinogen test can discriminate between acute DVT and post-thrombotic sequelae of a recent DVT. The ^{125}I -fibrinogen test will, according to the authors, be positive only in acute DVT because only in this situation is there an active incorporation of ^{125}I -fibrinogen into the thrombus. However, in the light of the present discussion it may be that the ^{125}I -fibrinogen test rather reflects circulatory changes secondary to the DVT than an active thrombus uptake, as was shown for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test. Since the latter test was not normalized until more than six months in patients with extensive DVT and thus, could not discriminate between an acute recurrent DVT and post-thrombotic sequelae, the usefulness of the ^{125}I -fibrinogen test in this situation must still be considered controversial.

Summary and conclusions

Deep venous thrombosis (DVT) is a common and potentially life-threatening disease. Clinical diagnosis of DVT is generally considered unreliable and phlebography has not been accepted as a suitable screening test due to side-effects, inconvenience and costs. Thus, there is a great need for simple diagnostic technique to help the attending physician in making the correct diagnosis.

Our interest in radionuclide tests started with an evaluation of $^{99}\text{Tc}^{\text{m}}$ -plasmin for diagnosis of established DVT (I). The $^{99}\text{Tc}^{\text{m}}$ -plasmin test showed a high sensitivity (100%) using phlebography as a reference method. Furthermore, the test is non-invasive, convenient to the patient, easy to perform and rapid (30 min). Thus, it is well suitable as a screening

test. However, the low specificity of the test necessitates phlebography in patients with a positive test. The benefit of using the $^{99}\text{Tc}^{\text{m}}$ - plasmin test is that already after 30 min, DVT can be reliably excluded in about 1/3 of the examined patients.

We tried to reduce the high number of falsely positive tests by evaluating new criteria for positive and negative tests (III). On the basis of maintaining the highest sensitivity possible (98-100 %) the two most efficient criteria increased the specificity to 66-69 %. Further increase in specificity could only be achieved at the cost of decreased sensitivity.

The $^{99}\text{Tc}^{\text{m}}$ - plasmin test was made repeatedly for up to twelve months in a group of patients with DVT and was found to remain positive up to more than six months after initial admission (IV). This indicates that the increased accumulation of radioactivity in the leg with DVT is probably not related to the acute thrombotic process. Furthermore, it also shows that a positive test should be evaluated with caution in patients with a history of recent DVT. The time to normalization of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test showed a considerable variation and was significantly related to the extent of the thrombus, as determined by phlebography.

In order to study the significance of any specific $^{99}\text{Tc}^{\text{m}}$ - plasmin uptake by the thrombus for the outcome of the plasmin test, we compared the distributions of $^{99}\text{Tc}^{\text{m}}$ - plasmin and $^{99}\text{Tc}^{\text{m}}$ - labeled erythrocytes, respectively, in the legs of patients with suspected DVT (II). The results showed similarly increased activity of the two radiopharmaceuticals in the diseased legs. Thus, when compared to $^{99}\text{Tc}^{\text{m}}$ - labeled erythrocytes, there was no increase in the uptake of $^{99}\text{Tc}^{\text{m}}$ - plasmin by the thrombus. Further comparison between $^{99}\text{Tc}^{\text{m}}$ - plasmin and $^{99}\text{Tc}^{\text{m}}$ - labeled erythrocytes, respectively, and also strain gauge plethysmography showed similar results of the three tests both in the acute phase and during six months' follow-up (V). These data suggest that the $^{99}\text{Tc}^{\text{m}}$ - plasmin test

reflects hemodynamic changes secondary to the DVT rather than a specific thrombus uptake.

The thrombus uptake of radiopharmaceuticals supposed to be actively involved in the thrombotic process (fibrinogen, plasmin, leukocytes and platelets) as well as radiopharmaceuticals without any known relation to this process (antimony colloid, tin-sulphur colloid, sulphur colloid, albumin microcolloid, albumin, erythrocytes, free and reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate) were studied in a rabbit model (VI). The accumulation of radioactivity in the thrombus was determined in vivo from scintillation camera images and in vitro by well counting, after dissection of the vein with the experimental thrombus. We found a low thrombus to blood ratio of specific activities for all the radiopharmaceuticals, after injection into the general circulation. The results exclude a high degree of specific interaction between any of the radiopharmaceuticals and the thrombus in our model.

The labeling efficiencies of the radiopharmaceuticals were monitored by gel chromatography column scanning. Our results showed high labeling efficiencies although less than 100 %. Lower labeling efficiencies were found when the resolving capacity of the control system was increased. This might introduce some uncertainty as to what component in the injected preparation that caused the activity uptake in the thrombus. Therefore, we used the 'radiochemical impurities' free and reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate as control substances when studying the thrombus uptake in the rabbit model. Since the control substances showed similar thrombus uptake as the other radiopharmaceuticals, this study underlined the importance of quality control for a correct understanding of what component of the radiopharmaceutical is monitored in the radionuclide tests.

One of the radiocolloids, $^{99}\text{Tc}^{\text{m}}$ - human serum albumin microcolloid (HSAC), was subsequently evaluated in patients with suspected DVT (VII). Although this substance has no known relation to coagulation or fibrinolysis, similar sensitivity and specificity were obtained as previously for $^{99}\text{Tc}^{\text{m}}$ - plasmin. These results suggested a common mechanism of action for the different radiopharmaceuticals, not related to any specific thrombus uptake.

Our results point to circulatory changes secondary to the established DVT as being most important for the outcome of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test and the $^{99}\text{Tc}^{\text{m}}$ -HSAC test. Furthermore, data presented in this thesis indicate that this may also apply to other radiopharmaceuticals including ^{125}I - fibrinogen.

The present study warrants a reappraisal of current radionuclide tests for diagnosis of DVT and the following conclusions are drawn.

Quality control of the radiopharmaceutical is essential for a correct understanding of the results.

Radiopharmaceuticals so far used in diagnosis of DVT are not taken up to a significant degree by the thrombus. Their distribution rather reflects hemodynamic changes secondary to the DVT.

Radiopharmaceuticals so far used can, however, still form the basis for radionuclide tests that are non-invasive, convenient to the patient, easy to perform and rapid.

Criteria for positive and negative tests should be chosen in such a way that the highest sensitivity possible is achieved. Consequently, a negative test will reliably exclude DVT.

By using non-specific radiopharmaceuticals, a low specificity is inherent in the test. In patients with a positive radionuclide test, phlebography is considered the method of choice to get a definitive diagnosis.

Radionuclide tests may remain positive for up to more than six months after the acute phase. Thus, the tests should be evaluated with caution in patients with a history of recent DVT. On the other hand, the tests may be useful in following the normalization of the hemodynamic changes secondary to acute DVT.

The development of new, more thrombus-specific radiopharmaceuticals will require additional knowledge about both the radiolabeling techniques and the thrombotic process.

Acknowledgements

I would like to express my sincere gratitude to

Per Ohlin, my advisor, who initiated this study and continuously provided expert guidance, generous support and ample laboratory facilities.

Bengt Lundh, my second advisor, who has always supported me and encouraged my interest in thrombosis and hemostasis, even beyond this study. Bengt also taught me a lot about writing and made innumerable improvements of my drafts.

Jan Nilsson, whose generous support and expert knowledge of phlebography were essential for this study.

Lena Andersson, Sven-Erik Strand and Freddy Ståhlberg, for generous guidance in radiophysics and fruitful collaboration.

Lars Brudin, whose computer skills and invaluable advice greatly contributed to parts of this study.

Finally, I want to thank my parents, Barbro and Gunnar Edenbrandt, and my brothers, Anders and Lars, for their warm support and encouragement in moments of setbacks.

This study has been supported by grants from Thorsten and Elsa Segerfalk's Foundation for Medical Research, Malmöhus Läns Landsting and the Swedish National Association against Heart and Chest Diseases.

References

- Adolfsson L, Nordenfelt I, Olsson H, Torstensson I (1982) Diagnosis of deep vein thrombosis with $^{99}\text{Tc}^{\text{m}}$ - plasmin. *Acta Med Scand* 211: 365-368
- Ahlbäck S, Bygdeman S, Waltz R (1977) The value of venous plethysmography in the diagnosis of venous thrombosis and for evaluation of therapeutic results. *Stand of Cardio-Angiological Methods* 4: 54-58
- Albrechtsson U, Olsson CG (1976) Thrombotic side-effects of lower limb phlebography. *Lancet* 1: 723-724
- Alkjaersig N, Fletcher AP, Sherry S (1959) The mechanism of clot dissolution by plasmin. *J Clin Invest* 38: 1086-1095
- Amris CJ, Larsen V, Mogensen B, Storm O (1963) Infusion of porcine plasmin in man. Studies on toxicology and pharmacodynamics. *Scand J Clin & Lab Invest* 15: 179-188
- Amris CJ, Amris A (1963) Investigations concerning activator-free porcine plasmin. *Scand J Clin & Lab Invest* 15: 189-197
- Amris A, Amris C (1964) Turnover and distribution of ^{131}I iodine-labelled human fibrinogen. *Thrombos Diathes haemorrh (Stuttg.)* 3/4: 404-422
- Amris CJ, Larsen V, Mogensen B, Storm O (1964) Turnover and distribution of ^{131}I - labelled porcine plasmin in man and dog. *Dan Med Bull* 11: 146-152
- Amris CJ (1966) Investigations concerning activator-free porcine plasmin. *Scand J Clin & Lab Invest, Suppl* 89: 1-23
- Amris CJ, Larsen V, Brøckner J (1966) Scheme for fibrinolytic treatment with activator-free porcine plasmin. *Scand J Clin & Lab Invest, Suppl* 89: 24-33
- Aoki N & Harpel PC (1984) Inhibitors of the fibrinolytic enzyme system. *Sem in Thromb & Hemost* 10 : 24 - 41
- Aronen HJ, Korppi-Tommola T, Suoranta HT, Taavitsainen MJ (1985) $^{99}\text{Tc}^{\text{m}}$ - plasmin test in deep vein thrombosis of the leg. *Eur J Nucl Med* 10: 10-12

Atkins P, Hawkins LA (1965) Detection of venous thrombosis in the legs. *Lancet* 2: 1217-1219

Atkins P, Hawkins LA (1968) The diagnosis of deep-vein thrombosis in the leg using ^{125}I - fibrinogen. *Br J Surg* 55: 825-830

Bardfeld PA, Rand J, Goldsmith SJ (1981) The use of technetium-99m sulfur colloid as a marker for experimental venous thrombosis. *J Nucl Med* 22: 598-600

Bardfeld PA, Harrison DA, Wasserstein G, Buetow G, Irwin GAL (1983) Detection of deep venous thrombosis with $^{99\text{m}}\text{Tc}$ - sulphur colloid. *Radiology* 146: 185-189

Bergvall U, Hjelmstedt Å (1968) Recanalization of deep venous thrombosis of the lower leg and thigh. *Acta Chir Scand* 134: 219-228

Bernstein K, Mattsson S, Ulmsten U, Åstedt B (1979) The ^{125}I - fibrinogen method for early diagnosis of venous thrombosis. *Vascular Surgery* 13: 33-41

Bernstein K, Jacobsson L, Mattsson S, Ulmsten U (1981) Depth localization of thrombi and dynamic studies of post-operative thrombosis using the ^{125}I - fibrinogen-sum-coincidence method. In: Thesis, Lund, Sweden

Beswick W, Cmiel R, Booth R, Vellar I, Gilford E, Chesterman CN (1979) Detection of deep venous thrombosis by scanning of $^{99\text{m}}\text{technetium}$ -labelled red-cell venous pool. *Br Med J* 1: 82-84

Browse NL, Clapman WF, Croft DN, Jones DJ, LeaThomas M, Olwen Williams J (1971) Diagnosis of established deep vein thrombosis with the ^{125}I -fibrinogen uptake test. *Br Med J* 4: 325-328

Browse NL (1972) The ^{125}I -fibrinogen uptake test. *Arch Surg* 104:160-163

Chandler AB (1958) In vitro thrombotic coagulation of the blood. *Lab Invest* 7: 110-114

Collen D, Verstraete M (1975) Molecular biology of human plasminogen. II. Metabolism in physiological and some pathological conditions in man. *Thromb Diath Haemorrh* 34: 403-408

Collen D, Wiman B (1979) Turnover of antiplasmin, the fast acting plasmin inhibitor of plasma. *Blood* 53: 313-324

Collen D (1980) On the regulation and control of fibrinolysis. Edward Kowalski Memorial Lecture. *Thromb Hemost.* 43: 77-89

Dahlborn M, Ahlborg G, Söderborg B, Virgin J, Wilczec B (1984) Gamma camera detection of ^{99m}Tc - plasmin in the diagnosis of deep-vein thrombosis. *Eur J Nucl Med*, 9, 499 - 501

Dahn I, Eiriksson E (1968) Plethysmographic diagnosis of deep venous thrombosis of the leg. *Acta Chir Scand*, Suppl 398: 33-42

Darte L, Persson RBR (1979) Gel chromatography column scanning (GCS) method of choice for quality control of ^{99m}Tc - plasmin preparations. *J Liq Chromatogr.* 2: 499-509

de Hevesy G (1964) Some applications of isotope indicators. In: Nobel Lectures Chemistry, pp. 9-41. Elsevier publishing company, Amsterdam

Flanc C, Kakkar VV, Clarke MB (1968) The detection of venous thrombosis of the legs using ^{125}I - labelled fibrinogen. *Br J Surg* 55: 742-747

Gomes AS, Webber MM, Buffkin D (1982) Contrast venography vs radionuclide venography; A study of discrepancies and their possible significance. *Radiology* 142: 719-728

Gomez RL, Brownell Wheeler H, Belko JS, Warren R (1963) Observations on the uptake of a radioactive fibrinolytic enzyme by intravascular clots. *Ann of Surg* 158: 905-911

Haeger K (1969) Problems of acute deep venous thrombosis. 1. The interpretation of signs and symptoms. *Angiology* 20: 219-223

Hallböök T, Göthlin J (1971) Strain gauge plethysmography and phlebography in diagnosis of deep venous thrombosis. *Acta Chir Scand* 137: 37-52

Harwig SSL, Harwig JF, Coleman RE, Welch MJ (1976) In vivo behavior of ^{99m}Tc - fibrinogen and its potential as a thrombus imaging agent. *J Nucl Med* 17:40-46

Havig Ö (1977) Deep vein thrombosis and pulmonary embolism. *Acta Chir Scand*; Suppl 478

Hayt DB, Blatt CJ, Freeman LM (1977) Radionuclide venography: Its place as a modality for the investigation of thromboembolic phenomena. *Sem Nucl Med* 3: 263-281

Hedner U, Johansson L, Nilsson IM (1978) Effects of porcine plasmin on the coagulation and fibrinolytic systems in humans. *Blood* 51: 157-164

Hirsh J, Hull R (1982) Natural history and clinical features of venous thrombosis. In: *Hemostasis and thrombosis: Basic principles and clinical practice* (eds. Colman RW, Hirsh J, Marder VJ, Salzman EW) pp 831-843. JB Lippincott Company, Philadelphia

Hobbs JT, Davies JWL (1960) Detection of venous thrombosis with ^{131}I -labelled fibrinogen in the rabbit. *Lancet* 2: 134-135

Hobbs JT (1962) External measurement of fibrinogen uptake in experimental venous thrombosis and other local pathological states. *Br J Exp Path* 43: 48-58

Hull R, Hirsh J (1982 a) Cost-effectiveness of non-invasive diagnosis of deep vein thrombosis in symptomatic patients. In: *Non-invasive techniques in vascular disease* (ed. E.F. Bernstein), pp. 560-569. C V Mosby Company, London.

Hull R, Hirsh J (1982 b) Diagnosis of venous thrombosis. In: *Hemostasis and thrombosis: Basic principles and clinical practice* (eds. Colman RW, Hirsh J, Marder VJ, Salzman EW) pp 844-856. JB Lippincott Company, Philadelphia

Husted SE, Kraemmer-Nielsen H, Krusell L, Fasting H, Østergaard-Nielsen B, Bruun-Pedersen J, Dalgaard E, Hvid Hansen H (1984) Deep vein thrombosis detection by $^{99\text{m}}\text{Tc}$ - plasmin test and phlebography. *Br J Surg* 71: 65-66

Jay R, Hull R, Carter C, Ockelford P, Buller H, Turpie AGG, Hirsh J (1984) Outcome of abnormal impedance plethysmography results in patients with proximal-vein thrombosis: frequency of return to normal. *Thromb Res* 36: 259-263

Kakkar VV, Howe CT, Flanc C, Clark MB (1969) Natural history of postoperative deep vein thrombosis. *Lancet* 2: 230

Kakkar V (1972) The diagnosis of deep vein thrombosis using the ^{125}I fibrinogen test. *Arch Surg* 104: 152-159

Kempi V, van der Linden W, von Scheele C (1974) Diagnosis of deep vein thrombosis with $^{99\text{m}}\text{Tc}$ - streptokinase: A clinical comparison with phlebography. *Br Med J* 4: 748-749

Kempi V, van der Linden W, von Scheele C (1976) Uptake of $^{99\text{m}}\text{Tc}$ -tripolyphosphate in thrombotic leg. *Nuklearmedizin* 15: 14-17

Kempi V, von Scheele (1976) Diagnosis of deep-vein thrombosis with sodium pertechnetate. *J Nucl Med* 17: 1096-1099

Kempi V, van der Linden W (1981) Diagnosis of deep vein thrombosis with in vivo $^{99\text{m}}\text{Tc}$ - labeled red blood cells. *Eur J Nucl Med* 6: 5-9

Khan O, Ell PJ, Cullum ID, Jarrit PH, Williams ES (1981) Clinical and pharmacologic studies with $^{99\text{m}}\text{Tc}$ -plasmin. In: *Progress in radiopharmacology* (ed. PH Cox), pp. 157-171, Elsevier/North Holland Biomedical Press, Amsterdam

Krohn KA, Knight LC (1977) Radiopharmaceuticals for thrombosis detection; Selection, Preparation and critical evaluation. *Sem Nucl Med* 3: 219-228

Larsen V, Amris CJ, Brøckner J, Storm O (1966) Fibrinolytic treatment with activator-free porcine plasmin. *Scand J Clin & Lab Invest* 18, Suppl 89: 34-43

LeaThomas M, MacDonald LM (1978) Complications of ascending phlebography of the leg. *Br Med J* 2: 317-318

Lückers AEG, Luth WJ, Teule GJJ, Huijgens PC, Heidendal GAK (1984) Diagnosis of deep venous thrombosis with $^{99\text{m}}\text{Tc}$ - plasmin using the gamma camera. *Eur J Nucl Med* 9: 282-283

May R, Nissl R (1979) *Die Phlebographie der unteren Extremität*. George Thieme Verlag, Stuttgart, FRG

McFarlane AS (1958) Efficient trace labeling of proteins with iodine. *Nature* 182: 53

McFarlane AS (1963) In vivo behavior of I^{131} -fibrinogen. *J Clin Invest* 42: 346-361

McIvor J, Andersson RD, Britt RP, Dovey P (1975) Comparison of I^{125} -labelled fibrinogen uptake and venography in the detection of recent deep-vein thrombosis in the legs. *Br J Radiol* 48:1013-1018

Moroi M, Aoki N (1976) Isolation and characterization of alpha 2 - anti plasmin inhibitor from human plasma. A novel proteinase inhibitor which inhibits activator-induced clot-lysis. *J Biol Chem* 251: 5956-5965

Müller T (1985) Quality control of commercial ^{99m}Tc - human albumin kits. *Eur J Nucl Med* 10: 551-553

Müllertz S (1956) Mechanism of action and effect of plasmin in blood. *Acta Physiologica Scandinavica*, Suppl. 130

Nanson EM, Palko PD, Dick AA, Fedoruk SO (1965) Early detection of deep venous thrombosis of the leg using I^{131} tagged human fibrinogen: A clinical study. *Ann Surg* 162: 438-445

Negus D, Pinto DJ, LeQuesne LP, Brown N, Chapman M (1968) I^{125} - labelled fibrinogen in the diagnosis of deep-vein thrombosis and its correlation with phlebography. *Br J Surg* 55: 835-839

Nicolaides AN, Olsson CG (1982) Applications of isotope technology to the clinical study of arterial and venous disease. The $[^{99m}Tc]$ plasmin test. In: *Noninvasive diagnostic techniques in vascular disease*. (ed. Bernstein EF) pp. 159-160. The CV Mosby Company, London

Olsson CG, Albrechtsson U, Darte L, Persson RBR (1979) $^{99}Tc^m$ -plasmin for rapid detection of deep vein thrombosis. In: *On diagnosis of deep vein thrombosis*. Thesis, Lund, Sweden

Olsson CG, Albrechtsson U (1980) A modified I^{125} -fibrinogen technique in suspected deep vein thrombosis. *Acta Med Scand* 207: 461-467

Olsson CG, Albrechtsson U, Darte L, Persson RBR (1982) Tc - 99m plasmin for rapid detection of deep vein thrombosis. *Nucl Med Commun* 3: 41-52

Ouchi H, Warren R (1962) Detection of intravascular thrombi by means of ^{131}I - labeled plasmin. *Surgery* 51: 42-49

Palko PD, Nanson EM, Fedoruk SO (1964) The early detection of deep venous thrombosis using ^{131}I - tagged human fibrinogen. *Can J Surg* 7: 215-226

Persson BRR, Kempf V (1975) Labeling and testing of $^{99\text{m}}\text{Tc}$ -streptokinase for the diagnosis of deep vein thrombosis. *J Nucl Med* 16: 474-477

Persson BRR, Darte L (1977) Labeling plasmin with technetium-99m for scintigraphic localization of thrombi. *Int J Appl Radiat Isot* 28: 97-104

Persson B, Olsson CG, Darte L, Strand, Bergqvist L, Ståhlberg F (1981) Preparation and testing of $^{99\text{m}}\text{Tc}$ - labelled plasmin for thrombus detection. In: *Progress in radiopharmacology* (ed. PH Cox) 2: 147-156. Elsevier/North Holland Biomedical Press, Amsterdam

Pettit WA, DeLand FH, Pepper GH, Blanton L (1978) Characterization of tin-technetium colloid in technetium - labeled albumin preparations. *J Nucl Med* 19: 387-392

Prentice AG, Lowe GDO, Forbes CD (1982) Diagnosis and treatment of venous thromboembolism by consultants in Scotland. *Br Med J* 285: 630-632

Rabinov K, Paulin S (1983) Venography of the lower extremities. In: *Abrams Angiography; Vascular and Interventional Radiology* (ed. H.L. Abrams) vol 3, pp. 1877-1922. Little, Brown & Company, Boston

Rhodes BA, Turaihi KS, Bell WR, Wagner HN (1972) Radioactive urokinase for blood clot scanning. *J Nucl Med* 13: 646-648

Ryo UY, Colombetti LG, Polin SG, Pinsky SM (1976) Radionuclide venography: Significance of delayed washout; visualization of the saphenous system. *J Nucl Med* 17: 590-595

Siegel ME, Malmud LS, Rhodes BA, Bell WS, Wagner HN (1972) Scanning of thromboemboli with ^{131}I - streptokinase. *Radiology* 103: 695-696

Sottrup-Jensen L, Clayes H, Zajdel M, Petersen TE, Magnusson S (1978) The primary structure of human plasminogen: isolation of two lysine-binding fragments and one 'mini'-plasminogen (M.W. 38,000) by elastase-catalyzed-specific limited proteolysis. In: Progress in chemical fibrinolysis and thrombolysis, vol 3 (eds. Davidsson JF, Rowan RM, Samama MM, Desnoyers PC), pp. 191-209, Raven Press, NY

Storm O (1961) In vitro thrombolysis. *Thromb Diath Haemorrh* 6, Suppl 288-292

Storm O, Larsen V, Mogensen B (1961) Plasmin therapy combined with anticoagulants in severe thrombo-embolic conditions. *Acta Chir Scand Suppl* 283: 315-321

Storm O, Ollendorf P, Drewsen E, Tang P (1974) Acute deep vein thrombosis treated with porcine plasmin. *Thromb Diath Hamorrh* 32: 468-482

Strandness DE, Sumner DS (1975) Hemodynamics for Surgeons. Grune & Stratton, Inc., New York

Sumner DS (1982) Strain gauge plethysmography, In: Non-invasive techniques in vascular disease (ed. EF Bernstein) pp. 468-481. C V Mosby Company, London

Tengborn L, Hedner U (1981) Demonstration of $^{99}\text{Tc}^{\text{m}}$ -labelled plasmin and α_2 -antiplasmin on the surface of ex vivo thrombi. In: Progress in fibrinolysis (eds. Davidson JF, Nilsson IM, Åstedt B) vol 5, pp. 324-328. Churchill Livingstone, London

Tengborn L, Hedner U, Rohlin M, Ståhlberg F (1982) Demonstration of $^{99\text{m}}\text{Tc}$ -labelled plasmin on the surface of ex vivo thrombi. *Thromb Res* 28: 783-791

Tjen HSLM (1981) Pathophysiology of thrombus - current clinical requirements for reagents for thrombus detection. In: Progress in radiopharmacology (ed. PH Cox) vol 2, pp. 103-118. Elsevier/North Holland Biomedical Press, Amsterdam

Vieras F, Barron EL, Parker GA, Grissom MP (1980) Experimental evaluation of Tc-99m sulphur colloid as a potential imaging agent in thromboembolic diseases. *J Nucl Med* 21: 723-728

Weir GJ, Roberts RC, Wenzel FJ, Sautter RD (1976) Visualization of thrombi with technetium - 99m urokinase. *Lancet* 2: 341-342

Wiman B, Collen D (1977) Purification and characterization of human antiplasmin, the fast-acting plasmin inhibitor in plasma. *Eur J Biochem* 78: 19-26

Young AE, Thomas ML, Browse NL (1974) Comparison between sequelae of surgical and medical treatment of venous thromboembolism. *Br Med J* 4: 127-130

Diagnosis of Deep Venous Thrombosis by Phlebography and $^{99}\text{Tc}^{\text{m}}$ -Plasmin

Carl-Magnus Edenbrandt, Jan Nilsson and Per Ohlin

From the Departments of Medicine, Diagnostic Radiology and Clinical Physiology, County Hospital, Helsingborg, Sweden

ABSTRACT. One hundred and thirty-four patients admitted to the medical emergency ward due to suspected deep venous thrombosis (DVT) were examined. The uptake of intravenously injected porcine $^{99}\text{Tc}^{\text{m}}$ -plasmin was estimated in both legs. Thereafter, phlebography was performed using a high osmolar contrast medium. All phlebographies were evaluated independently. All patients with negative phlebography were examined clinically after 3-5 days. The plasmin test and phlebography were repeated when called for. The sensitivity of the plasmin test was 100% and the specificity 51% when compared to phlebography. The extension of the DVT as demonstrated by the plasmin test was similar to that determined by phlebography. Post-phlebographic thrombosis was very rare. It is concluded that $^{99}\text{Tc}^{\text{m}}$ -plasmin test is a rapid method, convenient to the patient and well suitable as a screening test. The results indicate that a negative plasmin test excludes DVT while a positive test necessitates additional examination by phlebography.

Key words: deep venous thrombosis, diagnosis of deep venous thrombosis, post-phlebographic thrombosis, phlebography, $^{99}\text{Tc}^{\text{m}}$ -plasmin test.

Acta Med Scand 211: 59-64, 1982.

Received May 22, 1981.

Deep venous thrombosis (DVT) is a common disorder (19) and difficult to diagnose on the basis of symptoms and signs alone (21). Thus, laboratory tests are necessary for a correct diagnosis of DVT. The standard procedure is phlebography, which has some disadvantages. It is inconvenient and painful for the patient, it may cause thrombosis (1, 8, 24) and is expensive. Radionuclide methods have been used for nearly 20 years as alternatives to phlebography (6, 7, 14, 23), the most common being the ^{125}I -fibrinogen uptake test. It has a good sensitivity but may require up to 48 hours before the final result can be obtained (6, 15, 25, 27). Therefore, this method is not very useful in an emergency

ward. The $^{99}\text{Tc}^{\text{m}}$ -plasmin test has recently been reported to be a rapid and sensitive method for the diagnosis of DVT (30).

In the present study the $^{99}\text{Tc}^{\text{m}}$ -plasmin test was compared with phlebography in a series of consecutive patients with suspected DVT. Follow-up examinations of all patients were made to study the incidence of post-phlebographic thrombosis.

PATIENTS AND METHODS

One hundred and thirty-four patients, 60 males and 74 females, admitted to the medical emergency ward due to suspected DVT during weekdays from Dec. 1979 to Oct. 1980, were studied. Both $^{99}\text{Tc}^{\text{m}}$ -plasmin test and phlebography were performed within 24 hours. The plasmin test always preceded the phlebography.

If phlebography was positive for DVT, the patient was admitted for anticoagulant therapy, if not, other diagnostic possibilities were evaluated. All patients without DVT were re-examined after 3-5 days in order to discover any post-phlebographic thrombosis and to search for the most probable diagnosis. At this follow-up all patients were examined according to a specified protocol (Table I). The plasmin test was repeated in all patients with negative test at the first visit. If the slightest deterioration was suspected at the follow-up examination or the second plasmin test turned out to be positive, a second phlebography was performed on the same day. At the end of the study all phlebograms were re-evaluated independently by one of us (J. N.). The results of this re-evaluation were considered to be the definite outcome of the phlebographies.

$^{99}\text{Tc}^{\text{m}}$ -plasmin test. Porcine plasmin (Novo, Denmark) was kept in kits containing 123 CTA-u (about 10 mg) plasmin, 2 μmol $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 250 μmol NaCl and HCl to pH 2.0-3.0. The kits were stored at a temperature below -20°C . The $^{99}\text{Tc}^{\text{m}}$ -plasmin was prepared in the following way: 0.27 ml 0.1 N HCl was added to the kit, which was shaken a few times. Thereafter 1.0 ml $^{99}\text{Tc}^{\text{m}}$ -pertechnetate, about 300 MBq, (Radiochemical Centre, Amersham, England) in 0.9% NaCl was added and the vial was shaken

Correspondence to: Dr C.-M. Edenbrandt, Department of Medicine, Lasarettet, S-251 87 Helsingborg, Sweden.

Table I. Examination schedule used at follow-up 3–5 days after initial phlebography

Symptoms and signs in (A) 30 of 32 patients with negative plasmin test and negative phlebography, (B) 26 of 31 patients with positive plasmin test and negative phlebography (figures in parentheses indicate number of patients among those 4 who showed impairment at follow-up)

	No. of pats.	
	A	B
<i>Symptoms</i>		
No complaints	14	12
<i>Complaints</i>		
Pain	12	11 (4)
Tenderness	3	4 (3)
Swelling	8	6 (3)
Soreness	6	7 (3)
Fever	—	—
Impairment	—	4 (4)
<i>Signs</i>		
No pathological findings	16	11 (1)
<i>Pathological findings</i>		
Pain	10	6 (3)
Swelling of calf	8	14 (3)
Tenderness	2	4 (3)
Increase of temperature	1	4 (1)
Erythema	—	4 (—)
Dilatation of veins	4	1 (1)

until all plasmin was dissolved. By adding 0.9% NaCl, the volume was then adjusted to 3.5 ml. pH was controlled to be 2.0–2.2. Fresh $^{99}\text{Tc}^{\text{m}}$ -plasmin was prepared each morning and kept at room temperature for at least one hour before use. The labeling efficiency, i.e. the percentage of $^{99}\text{Tc}^{\text{m}}$ -pertechnetate taken up by plasmin, was estimated once a week by scanning of a gel chromatography column (Sephadex medium 25, Pharmacia, Sweden). The labeling efficiency was $91 \pm 2.5\%$ (mean $\pm$ S.D.); lowest value 86%.

The plasmin test was performed in the following way. Twelve points were marked on each leg. One foot point (on the medial malleolus), five points on the lower leg (equally distributed between the medial malleolus and the medial fissure of the knee), one knee point and five points on the upper leg (equally distributed between the medial fissure of the knee and the inguinal ligament). The most cranial point on the lower leg and the knee point were measured from behind. During the measurements, the patient was in a supine position with the legs elevated 45 degrees in order to minimize blood pooling. The leg points were always measured starting with the right foot point and subsequently alternating between the right and the left leg, in proximal direction.

About 20 MBq (0.1–1.5 ml) $^{99}\text{Tc}^{\text{m}}$ -plasmin were injected intravenously into an antecubital vein. The number of pulses at each leg point were estimated using a Thrombograph (Novo, Denmark). The apparatus consisted of a collimated NaI(Tl)-detector connected to a scaler-timer and a

Table II. Patients excluded from the initial series

Figures in parentheses indicate patients in whom not even the plasmin test could be carried out

N	Reason for exclusion
15	Incomplete phlebography due to failure to puncture a foot vein
5	Impossibility to interpret the phlebogram due to extreme post-thrombotic sequelae
4 (3)	Patient unable to co-operate due to senile dementia
2	Patient refused phlebography
1	History of allergy to contrast medium
1 (1)	Pregnancy
1	Re-evaluation of phlebogram not possible due to missing films
29 (4)	

printer. Before measuring the leg points, the time for 1×10^4 pulses over the forehead was determined. This time was used when measuring each leg point. The first measurement was started 5 min after the injection. If it was positive, no further measurements were performed. If it was negative, a second measurement was started 30 min after the injection. The test was considered negative or positive depending on the outcome of the second measurement. The number of pulses during the preset time at each leg point were tabulated. From the number of pulses at corresponding points of the right and left leg, the relative predominance of $^{99}\text{Tc}^{\text{m}}$ -plasmin uptake was calculated according to the following formula:

$$\frac{Dx}{Dx + Sin} \times 100 - 50.$$

Dx = no. of pulses at the right leg point, Sin = no. of pulses at the left leg point. This formula expresses by how many per cent the predominant leg exceeds the arithmetical mean value for corresponding leg points. The test was judged positive when three adjacent points of the suspected leg showed a predominant value ≥ 3 or if one point of the suspected leg showed a predominant value ≥ 13 (30).

Table III. Comparison between phlebography and $^{99}\text{Tc}^{\text{m}}$ -plasmin test for the diagnosis of DVT in 105 patients

$^{99}\text{Tc}^{\text{m}}$ -plasmin test	Phlebography (routine report)		Phlebography (definite outcome)	
	Positive	Negative	Positive	Negative
Positive	39	34	42	31
Negative	0	32	0	32

Table IV. Most probable diagnoses in (A) 30 of 32 patients with negative $^{99}\text{Tc}^m$ -plasmin test and negative phlebogram, (B) 26 of 31 patients with positive $^{99}\text{Tc}^m$ -plasmin test and negative phlebogram

	No. of pats.	
	A	B
Incompetent and/or varicose veins	7	3
Trauma	6	3
Unknown	5	4
Arthritis	4	4
Congestive heart failure	1	3
Thrombophlebitis, superficial	2	2
Erysipelas	—	3
Osteoarthritis of the hip	—	2
Hematoma (visible)	—	1
Baker's cyst	1	—
Muscle cramp	1	—
Skeletal pain	1	—
Rupture of meniscus	1	—
Polyneuropathy	1	—
Ergotamine intoxication	1	—

Phlebography. The method of phlebography described by Gullmo (18) was used in this study. It implies a dynamic two-step examination with injection of contrast medium into one foot vein and into the femoral vein in the groin. During the injection into the foot vein, the patient was sitting on a table with the feet resting on a footstool. A tourniquet was applied just above the ankle before puncturing. After the first exposure the tourniquet was removed and before the last exposures the foot was moved to contract the calf muscles. The amount of contrast medium, iothalamate meglumine (Conray Meglumine, Astra-Meditec, Sweden) with 282 mg iodine/ml, was 20–40 ml. Exposures were made over the foot-calf-knee region as well as over the thigh. During the injection into the femoral vein, the patient was lying supine and films were exposed during straining and at rest. Twenty ml of contrast medium were used at each exposure. Sometimes also an oblique projection was used in an effort to evaluate the deep femoral vein. All patients without signs of thrombosis were mobilized immediately after the examination.

RESULTS

Of the 134 patients who entered the study, 29 were excluded (Table II). The final series thus consists of 105 patients, 50 males and 55 females, with a mean age of 59.6 years (range 16–87). The first aim of the study was to compare the results of the $^{99}\text{Tc}^m$ -plasmin tests and the phlebograms. The results (regarding both the routine reports and the definite outcome of the phlebograms) appear from Table III. The $^{99}\text{Tc}^m$ -plasmin test showed a 100% sensitivity and a 51% specificity compared to phlebography.

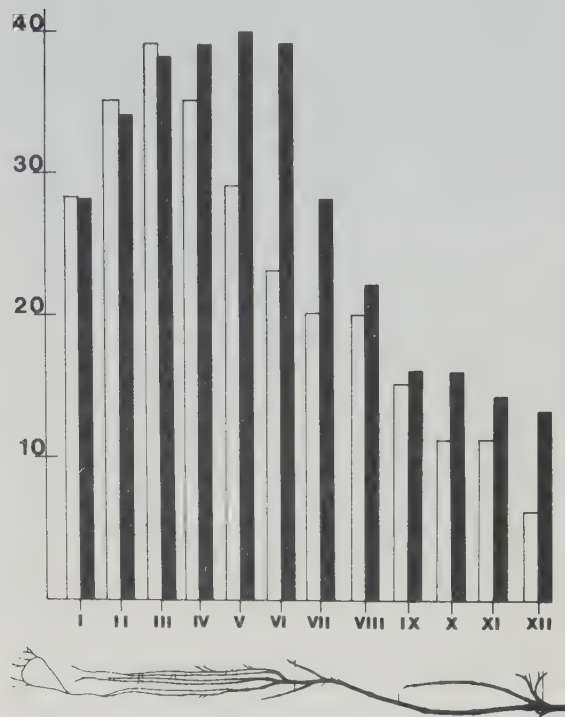


Fig. 1. Comparison between the localization and extension of DVT as judged from phlebography and $^{99}\text{Tc}^m$ -plasmin test in 42 patients. The thrombus extension in each patient was referred to the different leg points (I–XII). The total number of positive registrations in each leg point according to the plasmin test (□) and phlebography (■) are given.

Considering the definite outcome of the phlebograms, 42 (40%) of 105 patients had DVT. To examine the extension and localization of the DVT, the continuous positive leg points of the $^{99}\text{Tc}^m$ -plasmin test were tabulated and compared to the independently measured DVT extension from the phlebograms (Fig. 1). In 18 patients, the DVT was confined to the foot and calf region, that is, leg points 1–6, and was called distal thrombosis. In 24 patients, the DVT extended up to the knee joint and/or the upper leg, that is, leg points 7–12, and was called proximal thrombosis. In no patient was the thrombus confined to the femoral vein only. It should be noted that one of the 42 patients with DVT had been examined by phlebography 5 days before she entered this study. Her first phlebography was normal which means that she most likely had a post-phlebographic thrombosis. Otherwise, no serious adverse effects were noted during or after the phlebographies.

Follow-up of negative $^{99}\text{Tc}^{\text{m}}$ -plasmin tests. Thirty-two patients had a negative $^{99}\text{Tc}^{\text{m}}$ -plasmin test and a negative phlebogram. Thirty of these were re-examined clinically 3–5 days after the first phlebography and 28 underwent a second plasmin test. This test was still negative in 25 patients. Three patients with a positive second plasmin test were subjected to a second phlebography on the same day. All three phlebograms were negative. The symptoms and signs at the re-examination appear from Table I. All patients had improved during the observation period. The most probable diagnoses of the 30 patients re-examined are tabulated (Table IV). Four patients underwent no second plasmin test and two of these did not show up for re-examination.

Follow-up of falsely positive $^{99}\text{Tc}^{\text{m}}$ -plasmin tests. Thirty-one patients had a positive plasmin test but no DVT at the definite outcome of the phlebograms. The plasmin accumulation was confined to the foot and calf in 18 of them and extended up along the thigh in 13.

Five patients were not re-examined. One of them was diagnosed as having migrating thrombophlebitis and was referred for surgery, two were on anticoagulant therapy in spite of a negative phlebogram and two did not turn up. In 22 of the remaining 26 patients, symptoms and signs had clearly improved at the re-examination 3–5 days later (Table I). Their probable diagnoses appear from Table IV. Symptoms and signs had deteriorated in four patients (Table I). A second phlebography on the same day was still normal in all. The probable diagnoses of these four patients appear from Table IV.

DISCUSSION

As plasminogen/plasmin is involved in fibrinolysis, its potential as a specific thrombus-localizing agent should be apparent (4, 16, 20, 22, 31, 36). The exact mechanism of interaction between $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin and the thrombus is not completely clarified but different hypotheses have been put forward (4, 5, 10, 37).

Porcine plasmin has been shown to have a lytic effect on human DVT (35). It has also been reported that porcine plasmin could be labelled with $^{99}\text{Tc}^{\text{m}}$ -pertechnetate and still preserve its lytic activity and that $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin accumulated rapidly in the region of experimentally induced, preformed thrombi in rabbits (32).

Studies on $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin as a diagnostic test for detection of DVT in humans have been published recently (12, 30). We have adopted, with small modifications regarding leg points and leg elevation, the methodology and criteria proposed by Olsson et al. (30). In our hands the method showed a 100% sensitivity and 51% specificity compared to phlebography, i.e. figures similar to their experiences.

The method of phlebography applied in this study (18) visualizes the deep venous system although only a small volume of contrast medium (20–40 ml) is used for the distal injection. The dynamic way of performing the examination with contraction of the calf muscles also gives a good view of the flow of contrast medium through the calf and thigh. Sometimes the uppermost part of the femoral vein is poorly visualized, which in fact gives theoretical chance of missing thrombi in this part of the femoral vein. However, checking up all patients with negative phlebography rules out the existence of clinically relevant DVT. Disadvantages of phlebography are technical problems in puncturing a superficial vein of a swollen, edematous foot and difficulties in visualizing the deep femoral vein because of valve resistance to retrograde flow. Many reports of adverse reactions to phlebography, from minor discomfort to post-phlebographic thrombosis and skin necrosis, have been published (1, 8, 9, 24, 34, 38).

For various reasons, phlebography could not be performed properly in 23 of our 134 patients (Table II). At the final evaluation, 6 phlebograms from 5 patients showed extreme post-thrombotic changes. Whether a current DVT existed or not, could therefore not be confirmed with certainty. This means that phlebography could not be used for the final diagnosis of DVT in 28 of our 134 patients.

The routine report had diagnosed 39 of 42 patients with DVT at the definite outcome. In three patients the diagnosis of DVT was missed. One patient had multiple small thrombi in the deep foot veins plus a small thrombus in the fibular vein. Two patients had small thrombi distally in the popliteal vein. Thus, the routine report gave a 94% sensitivity and a 100% specificity compared to the final independent judgement. These figures are almost the same as those given by Olsson et al. (29).

DVT was found in 42 of our 105 consecutive patients, the incidence of DVT thus being 40%. In two other series (29, 30) of consecutive patients admitted to a medical emergency ward, the corre-

sponding figures were 50 and 34%, respectively. Distal thrombosis extending up to the knee joint was found in 18 patients and proximal thrombosis extending up along the thigh in 24 of our patients. The DVT localization and extension were similar to those previously found by others (29, 30).

Forty-two of 73 positive plasmin tests were truly positive. The localization and extension of the DVT as demonstrated by the plasmin test was similar to that determined by phlebography (Fig. 1). This may support the premise that plasmin is closely related to the DVT. Thirty-one of 73 positive plasmin tests were falsely positive. The most probable diagnoses of these patients appear from Table IV. This high rate of falsely positive plasmin tests is in accordance with that found by Olsson et al. (30). They proposed fibrin and/or fibrinogen accumulation as the common factor thus indicating a specific interaction between plasmin and fibrin/fibrinogen as the cause for accumulation. Other possibilities for a positive plasmin test such as a redistribution of blood due to the DVT may also be considered. Falsely positive tests are somewhat fewer than usually reported regarding the ^{125}I -fibrinogen uptake test (27, 29). The probable diagnoses in our series are in accordance with those suggested in connection with the ^{125}I -fibrinogen test (25, 29).

The possibility has been put forward that localized accumulation of the radionuclide in the thigh could represent a thrombus in the deep femoral vein not exposed by the phlebogram (28). In only two of our 31 falsely positive plasmin tests the accumulation of plasmin was confined only to the thigh. Both patients were completely recovered at the time of the re-examination and no clinical signs of DVT were then found. Thus, isolated DVT in the deep femoral vein seems to be rare.

The risk of post-phlebographic thrombosis is well known and several reports concerning this problem have been published (1, 8, 11). This complication has previously been considered rare (13). Recent reports have found thrombotic complications of phlebography to be more common (1, 3, 9, 38). Post-phlebographic thrombosis has been ascribed to the phlebographic method used, the osmolality of the contrast medium and/or the volume of contrast medium injected. Different ways have been described to avoid the complication, e.g. flushing with saline after phlebography and then applying a compression bandage (34), using non-ionic contrast media (2, 3, 38) or diluting the contrast medium (9).

We used an ionic contrast medium but the amount injected distally was limited to less than 40 ml. This and the fact that the calf muscles are contracted during the examination will guarantee that the contrast medium does not remain long in the calf veins.

Sixty-three of our 105 patients had an initially negative phlebogram. One patient had a post-phlebographic thrombosis already at the first examination but no further such thrombosis was found (drop-out rate 6%). Thus, when performing phlebography as described and using at most 40 ml of contrast medium in the distal injection, thrombotic complications have been rare in spite of an ionic contrast medium. The present method of phlebography (18) seems superior to other methods described (17, 33) with respect to the frequency of post-phlebographic thrombosis.

CONCLUSIONS

Phlebography is an expensive invasive method which is unpleasant and painful to the patient. It is therefore less suitable as a screening method for DVT. The plasmin test, on the contrary, is non-invasive, easy to perform, rapid and convenient to the patient. No adverse reactions have been found when more than 500 patients have been examined hitherto (26). Moreover, its good sensitivity makes it well suitable as a screening test for DVT. A positive plasmin test, however, necessitates an additional phlebography.

REFERENCES

1. Albrechtsson, U. & Olsson, C. G.: Thrombotic side-effects of lower limb phlebography. *Lancet* 1: 723-724, 1976.
2. — Thrombosis following phlebography with ionic and non-ionic contrast media. *Acta Radiol (Diagn)* 20: 46-52, 1979.
3. — Thrombosis after phlebography: A comparison of two contrast media. *Cardiovasc Radiol* 2: 9-18, 1979.
4. Alkjaersig, N., Fletcher, A. P. & Sherry, S.: The mechanism of clot dissolution by plasmin. *J Clin Invest* 38: 1086-1095, 1959.
5. Ambrus, C. M. & Markus, G.: Plasmin-antiplasmin complex as a reservoir of fibrinolytic enzyme. *Am J Physiol* 199: 491-494, 1960.
6. Atkins, P. & Hawkins, L. A.: The diagnosis of deep vein thrombosis in the leg using ^{125}I -fibrinogen. *Br J Surg* 55: 825-830, 1968.
7. Beswick, W., Chmiel, R., Booth, R., Vellar, I., Gilford, E. & Chesterman, C. N.: Detection of deep venous thrombosis by scanning of $^{99\text{m}}$ technetium-labelled red-cell venous pool. *Br Med J* 1: 82-84, 1979.

8. Bettman, M. A. & Paulin, S.: Leg phlebography: The incidence, nature and modification of undesirable side effects. *Radiology* 122: 101–104, 1977.
9. Bettman, M. A., Salzman, E. W., Rosenthal, D. et al.: Reduction of venous thrombosis complicating phlebography. *AJR* 134: 1169–1172, 1980.
10. Chesterman, C. N., Allington, M. J. & Sharp, A. A.: Relationship of plasminogen activator to fibrin. *Nature New Biol* 238: 15–17, 1972.
11. Cranley, J. J.: Venous thrombosis secondary to phlebography. In: *Vascular surgery*, vol. 2, pp. 70–71. Harper & Row, Hagerstown 1975.
12. Deacon, J. M., Ell, P. J., Anderson, P. & Khan, O.: Technetium 99m-plasmin: a new test for the detection of deep vein thrombosis. *Br J Radiol* 53: 673–677, 1980.
13. Editorial comments. *Cardiovasc Radiol* 2: 15–18, 1979.
14. Fenech, A., Dendy, P. P., Hussey, J. K., Bennett, B. & Douglas, A. S.: Indium-111 labelled platelets in diagnosis of leg-vein thrombosis: preliminary findings. *Br Med J* 280: 1571–1573, 1980.
15. Flanc, C., Kakkar, V. V. & Clarke, M. B.: The detection of venous thrombosis of the legs using ¹²⁵I-labelled fibrinogen. *Br J Surg* 55: 742–747, 1968.
16. Gomez, R. L., Wheeler, H. B., Belko, J. S. & Warren, R.: Observations on the uptake of a radioactive fibrinolytic enzyme by intravascular clots. *Ann Surg* 158: 905–911, 1963.
17. Greitz, T.: The technique of ascending phlebography of the lower extremity. *Acta Radiol* 42: 421–441, 1954.
18. Gullmo, Å.: On the technique of phlebography of the lower limb. *Acta Radiol* 46: 603–620, 1955.
19. Harvig, Ö.: Deep vein thrombosis and pulmonary embolism. *Acta Chir Scand (Suppl)* 478, 1977.
20. Harwig, S. S. L., Harwig, J. F., Sherman, L. A., Coleman, R. E. & Welch, M. J.: Radioiodinated plasminogen: An imaging agent for pre-existing thrombi. *J Nucl Med* 18: 42–45, 1977.
21. Haeger, K.: Problems of acute deep venous thrombosis. I. The interpretation of signs and symptoms. *Angiology* 20: 219–223, 1969.
22. Hedner, U., Johansson, L. & Nilsson, I. M.: Effects of porcine plasmin on the coagulation and fibrinolytic systems in humans. *Blood* 51: 157–164, 1978.
23. Kempf, V., van der Linden, W. & von Scheele, C.: Diagnosis of deep vein thrombosis with ⁹⁹Tc^m-streptokinase: A clinical comparison with phlebography. *Br Med J* 4: 748–749, 1974.
24. Lea Thomas, M. & MacDonald, L. M.: Complications of ascending phlebography of the leg. *Br Med J* 2: 317–318, 1978.
25. Negus, D., Pinto, D. J., LeQuesne, L. P., Brown, N. & Chapman, M.: ¹²⁵I-labelled fibrinogen in the diagnosis of deep-vein thrombosis and its correlation with phlebography. *Br J Surg* 55: 835–839, 1968.
26. Ohlin, P.: Personal communication, 1980.
27. Olsson, C. G.: A modified ¹²⁵I-fibrinogen technique for thrombus detection in the whole leg. *Scand J Clin Lab Invest* 39: 677–684, 1979.
28. — Disadvantages of phlebography. In: *Thesis*, Lund 1979.
29. Olsson, C. G. & Albrechtsson, U.: A modified ¹²⁵I-fibrinogen technique in suspected deep vein thrombosis. A comparison with plethysmography and phlebography. In: *Thesis*, Lund 1979.
30. Olsson, C. G., Albrechtsson, U., Darte, L. & Persson, R. B. R.: ⁹⁹Tc^m-plasmin for rapid detection of deep vein thrombosis. In: *Thesis*, Lund 1979.
31. Ouchi, H. & Warren, R.: Detection of intravascular thrombi by means of ¹³¹I-labeled plasmin. *Surgery* 51: 42–49, 1962.
32. Persson, R. B. R. & Darte, L.: Labeling plasmin with technetium-99m for scintigraphic localization of thrombi. *Int J Appl Radiat Isot* 28: 97–104, 1977.
33. Rabinov, K. & Paulin, S.: Roentgen diagnosis of venous thrombosis in the leg. *Arch Surg* 104: 134–144, 1972.
34. Richter, E. I. & Zeitler, E.: Die Röntgendiagnostik der akuten Phlebothrombose. *Radiologe* 20: 426–433, 1980.
35. Storm, O., Ollendorff, P., Drewsen, E. & Tang, P.: Acute deep vein thrombosis treated with porcine plasmin. *Thromb Diath Haemorrh* 32: 468–482, 1974.
36. Strachan, C. J. L., Scully, M. F. & Kakkar, V. V.: The behaviour of isotope labelled blood proteins in thrombosis. *Thromb Res* 4: 303–318, 1974.
37. Tengborn, L. & Hedner, U.: Demonstration of ⁹⁹Tc^m-labelled plasmin complexed with α_2 -antiplasmin (α_2 AP) on the surface of ex vivo thrombi. In: *Progress in fibrinolysis and thrombolysis*, vol. 5. Churchill Livingstone, Edinburgh. In press 1981.
38. Walters, H. L., Clemenson, J., Browse, N. L. & Thomas, M. L.: ¹²⁵I-fibrinogen uptake following phlebography of the leg. *Radiology* 135: 619–621, 1980.

Comparison between $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin and $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes in diagnosis of deep vein thrombosis

Carl-Magnus Edenbrandt, Jan Anders Dahlström, Jan Nilsson and Per Ohlin

Departments of Medicine, Clinical Physiology and Diagnostic Radiology, Central Hospital, Helsingborg, Sweden

(Received 15 September 1983; accepted 22 December 1983)

Summary. In 20 patients with suspect deep venous thrombosis (DVT), scintillation detector measurements were performed over each leg during the first 60 min after intravenous injection of $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin. Thereafter, $^{99}\text{Tc}^{\text{m}}$ -labelled autologous erythrocytes were injected i.v. and repeat measurements were performed. Finally, scintillation camera images of both legs were obtained. Phlebography was used as a reference method. A close relationship ($P < 0.01$) was found between the scintillation detector measurements, both in patients with DVT ($n=11$) and in patients without DVT ($n=9$). Thus, $^{99}\text{Tc}^{\text{m}}$ -plasmin is not specifically bound to the thrombus. Rather the clinical utility of the test depends mainly on circulatory changes secondary to the thrombus. Scintillation camera images of $^{99}\text{Tc}^{\text{m}}$ -erythrocytes in the legs were not useful for diagnosis of DVT in the calves but showed a high specificity for DVT in the popliteal and femoral veins.

Introduction

A simple, non-invasive and rapid screening method for deep venous thrombosis (DVT) in the lower extremities (Havig, 1977) would be desirable. During the last 2 years, the $^{99}\text{Tc}^{\text{m}}$ -plasmin test has shown a high diagnostic sensitivity in consecutive patients admitted to a medical emergency ward with phlebography as a reference method (Olsson, Albrechtsson, Darte & Persson, 1982; Edenbrandt, Nilsson & Ohlin, 1982; Adolfsson *et al.*, 1982). The main drawback of the test is its low specificity. Approximately half of the patients with a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test do not have DVT. Falsely positive $^{99}\text{Tc}^{\text{m}}$ -plasmin tests have been observed secondary to arthritis, incompetent or varicose veins, trauma, erysipelas, congestive heart failure, and changes related to old thrombosis (Edenbrandt *et al.*, 1982; Adolfsson *et al.*, 1982).

Correspondence: Dr Carl-Magnus Edenbrandt, Department of Medicine, Central Hospital, S-251 87 Helsingborg, Sweden.

The mechanism causing accumulation of radioactivity in the diseased leg after $^{99}\text{Tc}^{\text{m}}$ -plasmin injection has not yet been elucidated. *In vitro* $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin has been shown to adsorb to clot surfaces (Tengborn & Hedner, 1981). After intravenous injection, porcine plasmin circulates in the blood bound to α_2 -antiplasmin and α_2 -macroglobulin but it still demonstrates proteolytic activity with fibrinogenolysis (Hedner, Johansson & Nilsson, 1978). Our working hypothesis has been that a fraction of the injected $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin accumulates specifically at the thrombus site while the rest reflects circulating blood. An increase in local blood volume might explain the increased count rate in the diseased leg in patients with falsely positive tests.

In the present study, we have tried to reduce the number of falsely positive $^{99}\text{Tc}^{\text{m}}$ -plasmin tests by performing a measurement reflecting the local blood pool using $^{99}\text{Tc}^{\text{m}}$ -labelled autologous erythrocytes. By comparing the results of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test and the blood pool test, we hoped to determine whether an accumulation of $^{99}\text{Tc}^{\text{m}}$ -plasmin in the diseased leg was due to an increased blood volume, a changed blood-detector geometry or a specific binding to the thrombus site.

Materials and methods

PATIENTS

Twenty patients, 10 women and 10 men, with a mean age of 62.5 (range 41–78) years, admitted to the medical emergency ward with suspect DVT were included in the study. Clinical examination and $^{99}\text{Tc}^{\text{m}}$ -plasmin test were performed at admission. Patients with a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test were asked to participate in further investigations with $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes. Phlebography of the diseased leg was always made after the radionuclide studies on the same day. The present investigation was sanctioned by the research ethical committee, Faculty of Medicine, University of Lund.

$^{99}\text{Tc}^{\text{m}}$ -PLASMIN TEST

The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was performed in a standardized way as described previously (Edenbrandt *et al.*, 1982). Twelve points were marked on each leg including one foot point (on the medial malleolus), five points on the lower leg (equally distributed between the medial malleolus and the medial fissure of the knee), one knee point and five points on the upper leg (equally distributed between the medial fissure of the knee and the inguinal ligament). During the measurements the patient was in a supine position with the legs elevated 45° in order to minimize blood pooling. The leg points were always measured starting with the right foot point and subsequently alternating between the right and the left leg moving in the proximal direction. $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin (Lysofibrin, NOVO A/S, Denmark) was injected into an antecubital vein. The mean ($\pm$ SD) $^{99}\text{Tc}^{\text{m}}$ -plasmin activity administered was 21.0 ($\pm$ 1.7)

MBq. The count rate at each leg point was measured using a collimated NaI(Ti)-detector (Thrombograph, NOVO A/S, Denmark). Before measuring the leg points, the time for 10^4 pulses over the forehead was determined and used when measuring each leg point. The first measurement of all leg points was started 5 min after the injection, the second measurement was performed 30 min after injection. The criteria for positive and negative tests were adopted from earlier experiments by Olsson *et al.* (1982). The count rate at each leg point recorded during the present time was tabulated. From the count rate at corresponding points of the right and left leg, the relative predominance (R) of the $^{99}\text{Tc}^{\text{m}}$ -plasmin uptake was calculated and expressed in quotient units according to the following formula:

$$R = \frac{\dot{N}(Dx)}{\dot{N}(Dx) + \dot{N}(\text{Sin})} \times 100 - 50,$$

$\dot{N}(Dx)$ = count rate at the right leg point, $\dot{N}(\text{Sin})$ = count rate at the left leg point.

The test was considered positive, when three adjacent pairs of points showed $R \geq 3$ quotient units and/or when one pair of points showed $R \geq 13$ quotient units, provided that the suspected leg obtained the highest count rates, at five and/or 30 min after the injection. If the patient was included in the study, a third measurement of all leg points was performed at 60 min after the injection.

BLOOD POOL TEST

Prior to the $^{99}\text{Tc}^{\text{m}}$ -plasmin test 2 ml of blood was withdrawn from the patient, anticoagulated with heparin, and put into a sterile and evacuated vial. Three millilitres of sterile, pyrogen-free, 0.9% sodium chloride solution under nitrogen atmosphere was used to dissolve a freeze-dried, sterile and pyrogen-free mixture of 0.01 mg stannous chloride and 0.67 mg sodium pyrophosphate (TCK-II, CIS, France). One and a half millilitres of this kit solution was added to the blood sample. After incubation for 5 min, the blood sample was centrifuged at 650 g for 5 min and the supernatant plasma was discarded. After an additional wash with 0.9% sodium chloride, 0.8–1.4 ml (about 600 MBq) of $^{99}\text{Tc}^{\text{m}}$ -pertechnetate in saline was added to the erythrocytes. After incubation for 5 min, the erythrocytes were washed again. The labelling efficiency was determined in a 75 μl sample by separating erythrocytes from the saline phase in a micro hematocrit centrifuge and determining the count rate of each part in a dose calibrator (CRC-IORB, Capintec inc., U.S.A.).

Immediately after having completed the last measurement of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test, the $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes were injected into an antecubital vein. The mean ($\pm$ SD) $^{99}\text{Tc}^{\text{m}}$ -erythrocyte activity administered was 432 ($\pm$ 62) MBq. Five min after injection the number of counts during 1 s was measured at each leg point. Dead time corrections were performed. Only one measurement was performed on each leg

point. The results were calculated and presented in the same way as for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test.

Thereafter the patient stood on a foot-stool in front of a scintillation camera (Pho/Gamma LFOV, Siemens, U.S.A.) with a low-energy, all purpose collimator. The scintillation camera was connected to a digital system (Scintiview, Siemens, U.S.A.). The first image was taken as an anterior view of both thighs, the second image as a posterior view of both knee regions and the third image as a posterior view of both lower legs.

The exposure time was 180 s and there were about 2000 counts/cm² over the veins. These images were evaluated by one of the authors (P. O.), who was unaware of the outcome of the other tests. It was first determined if the main vessels, i.e. the popliteal and femoral veins, appeared normal or if any well-defined defect of the vessels could be seen. The count rate over any defect of the vessel was compared with the corresponding part of the vessel in the healthy leg to obtain a numerical grading. In addition P. O. looked for local accumulation of radioactivity suggestive of DVT, 'hot spots', and increased radioactivity in collateral vessels.

In order to monitor the stability of the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex and the $^{99}\text{Tc}^{\text{m}}$ -plasmin/ $^{99}\text{Tc}^{\text{m}}$ -erythrocyte relation *in vivo*, four blood samples were withdrawn from the patient. One sample (A) was drawn just before the injection of $^{99}\text{Tc}^{\text{m}}$ -erythrocytes, one (B) before and one (C) after measuring the leg points and finally one (D) after having obtained the scintillation camera images. The $^{99}\text{Tc}^{\text{m}}$ -activity was determined in 0.5 ml whole blood and, after centrifugation, in 0.5 ml plasma using a well counter (MR-1032, W+W electronic, Switzerland). All blood samples from the patient were counted at the same time and the count rates were corrected to the time of injection. Micro haematocrit was determined in all blood samples and the labelling efficiency was subsequently calculated.

PHLEBOGRAPHY

Phlebography was carried out using injection of contrast medium (Conray Meglumine, Astra-Meditec, Sweden) into a foot vein and into the common femoral vein in the groin (Gullmo, 1956). All phlebograms were re-evaluated by one of us (J. N.) who was unaware of the outcome of the other tests.

RADIATION DOSIMETRY

The estimated effective dose equivalents were 0.3 mSv for $^{99}\text{Tc}^{\text{m}}$ -plasmin (Persson *et al.*, 1981), 3.0 mSv for $^{99}\text{Tc}^{\text{m}}$ -erythrocytes (Malamud, 1978) and 1.5 mSv for phlebography (Edenbrandt, *et al.*, 1983).

STATISTICAL METHODS

The data were analysed using paired *t*-test, unpaired *t*-test with unequal variances,

F-test after linear regression and Chi-square test (Armitage, 1980). A difference was considered significant when $P < 0.05$. The data are given as mean $\pm$ SD unless otherwise stated.

Results

LABELLING CONTROL

The median labelling efficiency of the $^{99}\text{Tc}^{\text{m}}$ -erythrocytes *in vitro* was 98.6% (range 95.5–99.3). Blood samples withdrawn prior to injection of the $^{99}\text{Tc}^{\text{m}}$ -erythrocytes (A) and 3–5 min after the injection (B) were used to calculate how much of the total activity in B originated from $^{99}\text{Tc}^{\text{m}}$ -plasmin and $^{99}\text{Tc}^{\text{m}}$ -erythrocytes, respectively. On the assumption that the activity due to the $^{99}\text{Tc}^{\text{m}}$ -plasmin injection did not change between blood samples A and B (Khan *et al.*, 1981), the activity originating from $^{99}\text{Tc}^{\text{m}}$ -plasmin constituted $1.1 \pm 0.3\%$ of the total activity in blood sample B. Out of the total $^{99}\text{Tc}^{\text{m}}$ -activity found in blood sample C, obtained 5–10 min after the injection, and in blood sample D obtained 10–47 min after the injection, $94.8 \pm 1.4\%$ and $95.5 \pm 1.2\%$, respectively, were found in the erythrocyte fraction. Blood sample D contained $101.1 \pm 8.1\%$ of the total $^{99}\text{Tc}^{\text{m}}$ -activity found in blood sample B.

CLINICAL EVALUATION

On admission, patients were evaluated for symptoms and signs such as pain, tenderness, swelling of calf (> 1 cm compared with the controlateral leg), fever, erythema, dilatation of veins and Homans' sign. There was no statistically significant difference in the frequencies of these findings between patients with DVT and patients with falsely positive $^{99}\text{Tc}^{\text{m}}$ -plasmin tests.

SCINTILLATION DETECTOR MEASUREMENTS

The results of the measurements obtained at 5, 30 and 60 min after intravenous injection of $^{99}\text{Tc}^{\text{m}}$ -plasmin and at 5 min after injection of $^{99}\text{Tc}^{\text{m}}$ -erythrocytes were compared in each patient as shown for one patient in Fig. 1. In order to quantify any difference between the four measurements, the area under each curve was calculated. No statistically significant difference in total area under the curve was found between any of the four measurements when considering all patients or when considering only patients with DVT. On the contrary, a close relationship was found between measurements obtained at 5 min and 30 min after $^{99}\text{Tc}^{\text{m}}$ -plasmin injection ($P < 0.01$), as well as between 5 min and 60 min ($P < 0.01$), 30 min and 60 min ($P < 0.01$) and any of the measurement obtained 5, 30 and 60 min after the $^{99}\text{Tc}^{\text{m}}$ -plasmin injection compared with the results of the blood pool test ($P < 0.01$). These results were consistent even when considering only patients with or without DVT. When considering only that part of the curve exceeding three quotient units in the diseased

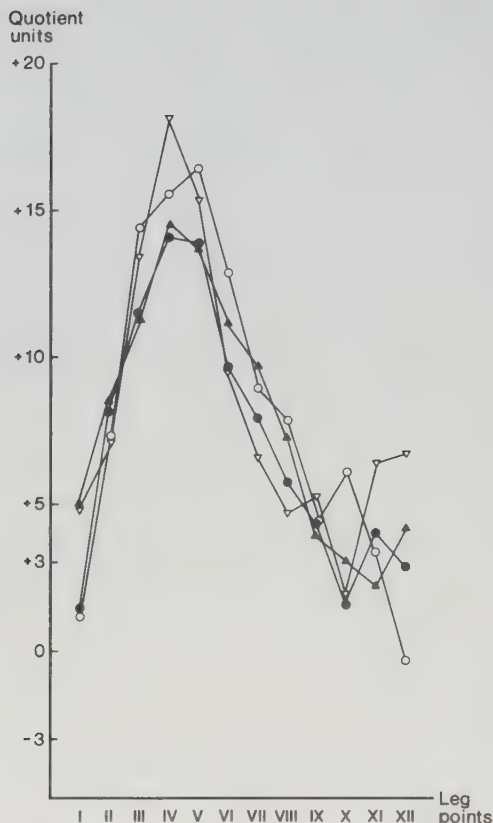


Fig. 1. The relative predominance of radioactivity in the diseased leg compared with the healthy leg expressed in quotient units, according to Olsson *et al.* (1982), at each leg point. Measurements of all leg points were made at 5 min (●), 30 min (▲) and 60 min (○) after injection of $^{99}\text{Tc}^{\text{m}}$ -plasmin and at 5 min (▽) after injection of $^{99}\text{Tc}^{\text{m}}$ -erythrocytes. Phlebography showed thrombosis extending from leg point I to X.

leg, calculated according to Olsson *et al.* (1982), no statistically significant difference was found between the area under the curve calculated for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test at 5 min, 30 min and 60 min and the blood pool test considering all patients or only patients with or without DVT. On the contrary, a close relationship was found between the areas obtained at 5, 30 and 60 min, respectively, after the $^{99}\text{Tc}^{\text{m}}$ -plasmin injection and the blood pool test ($P < 0.01$), both when considering all patients and when studying only patients with DVT or only patients without DVT.

The results of the four leg point measurements were compared with the extensions of the thrombi, as shown by phlebography. A greater number of positive test results were found at leg points over the thrombus and a greater number of negative tests were found distally as well as proximally to the thrombus at 5 min ($P < 0.05$), 30 min ($P < 0.01$) and 60 min after the $^{99}\text{Tc}^{\text{m}}$ -plasmin injection and at 5 min after the

$^{99}\text{Tc}^{\text{m}}$ -erythrocyte injection. When comparing the four measurements with each other, no statistically significant difference was found in the distribution of positive and negative test results. Considering only leg points over the thrombus, no statistically significant difference was found between the number of positive and number of negative test results in any of the four measurements. However, considering only leg points outside the thrombus, all measurements showed a greater number of positive test results obtained distally to the thrombus compared to results obtained proximally to the thrombus ($P<0.05$).

The count rates due to $^{99}\text{Tc}^{\text{m}}$ -plasmin were assumed not to change from the 60-min measurement until 5 min after the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte injection. We then found the count rates originating from $^{99}\text{Tc}^{\text{m}}$ -plasmin to constitute $3.1\pm0.8\%$ of total count rates determined after the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte injection (Fig. 2), while the activity in blood originating from $^{99}\text{Tc}^{\text{m}}$ -plasmin constituted only $1.1\pm0.3\%$ of the total blood activity, as shown above ($P<0.001$).

SCINTILLATION CAMERA IMAGES AND PHLEBOGRAPHY

Eleven patients out of 20 had DVT according to phlebography. Four patients had distal thrombosis, i.e. corresponding to leg points I–VI, and seven patients had proximal thrombosis, i.e. thrombus extension up to leg points VII–XII. Scintillation camera images showed well-defined defects of the femoral and/or the popliteal veins in the suspected leg of four patients, all of whom had proximal DVT. The defects in the images showed 15–26% less count rate compared with the corresponding part of

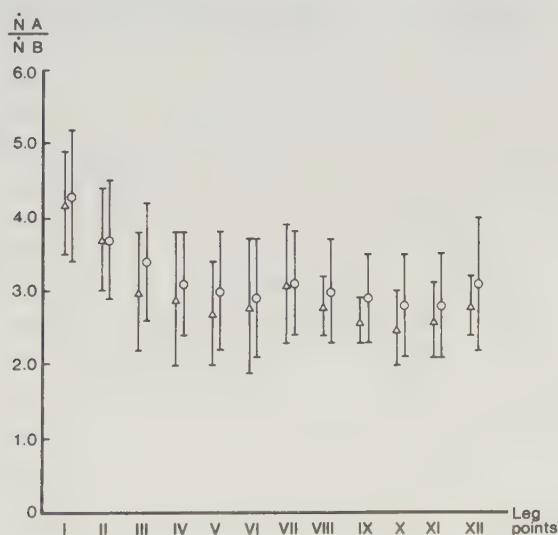


Fig. 2. The relationship in per cent between count rates determined 60 min after $^{99}\text{Tc}^{\text{m}}$ -plasmin injection (NA) and 5 min after $^{99}\text{Tc}^{\text{m}}$ -erythrocyte injection (NB) at each leg point in 11 patients with DVT (○) and nine patients without DVT (△). The data of each group are given as mean ($\pm$ SD).

the vein in the other leg. Three patients with proximal thrombosis and four patients with distal thrombosis had no defects in their images. In the contralateral leg, the images of three patients showed defects in main vessels. The count rates over the defects were 15–27% less than those obtained over corresponding sites in the other leg. Marked collateral vessels in the suspected leg were found in the images of four patients with proximal DVT and in one patient without DVT. Three patients with proximal thrombosis and four patients with distal thrombosis showed no collateral vessels in their images. Two patients showed collateral vessels in the images of the contralateral leg. Increased accumulation of radioactivity in localized areas of the calf suggestive of DVT, 'hot spots', were found in the images of the suspected leg in three patients. According to phlebography one of the patients had distal thrombosis and one had findings suggestive of the post-thrombotic state. Phlebography was normal in the third patient. Seven patients with proximal thrombosis and three patients with distal thrombosis showed no 'hot spots' in their images. In the contralateral leg, 'hot spots' were found in four patients. The images of five patients showed no defect in the main vessels, no marked collateral vessels and no 'hot spots' in either leg even though one of the patients had a proximal DVT and another one a distal DVT according to the results of the phlebography of the suspected leg.

Discussion

A specific interaction between $^{99}\text{Tc}^{\text{m}}$ -plasmin and the thrombus has been suggested as the basis for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test used for the diagnosis in DVT (Nicolaidis & Olsson, 1982). A serious drawback of the test is the considerable number of falsely positive tests. Its main advantage is the fact that a negative test excludes DVT with a high degree of probability (Edenbrandt *et al.*, 1982). In the present study $^{99}\text{Tc}^{\text{m}}$ -plasmin and $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes were compared in an attempt to elucidate the mechanism of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test. Our hypothesis was that by correcting the $^{99}\text{Tc}^{\text{m}}$ -plasmin activity for that reflecting circulating blood, calculated from the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte distribution, we should be able to get an estimation of the $^{99}\text{Tc}^{\text{m}}$ -plasmin 'bound' to the thrombus. Contrary to our expectations, we found almost identical curves for the distribution of the total $^{99}\text{Tc}^{\text{m}}$ -plasmin and the total $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex activities. The amount of $^{99}\text{Tc}^{\text{m}}$ -plasmin bound specifically to the thrombus must be negligible. Therefore the results presented in this study suggest that the clinical usefulness of this test depends mainly on circulatory changes secondary to the thrombosis.

Comparison between $^{99}\text{Tc}^{\text{m}}$ -erythrocytes, which were shown to remain intravascularly during the observation time, and $^{99}\text{Tc}^{\text{m}}$ -plasmin indicated an appreciable extravascular distribution of $^{99}\text{Tc}^{\text{m}}$ -plasmin in the legs. However, in all patients, this increased activity was mainly over the ankle and the knee region. No relation was seen between this increase in activity and the localization of the thrombus. The distal localization of the activity uptake suggest an affinity to bone tissue.

Labelling of erythrocytes with $^{99}\text{Tc}^{\text{m}}$ was performed *in vitro* because this method facilitates control of the efficiency of the labelling of the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex. The labelling efficiency depends on several factors such as the reductive capacity of the stannous ion in the kit (Smith & Richards, 1976; Huberty & Hattner, 1982), the total amount of Tc atoms added to the kit and the $^{99}\text{Tc}^{\text{m}}/^{99}\text{Tc}$ ratio (Smith *et al.*, 1976). Furthermore, the stability of the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex in the blood depends on the presence of reducing and oxidizing agents and degree of damage to the erythrocytes (Hladik, Nigg & Rhodes, 1982). In other studies we have identified several pitfalls which interfere with the formation of a stable $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex with a high degree of labelling efficiency. In the present study the labelling efficiency was found to be about 98%. Furthermore, we found about 95% of the total $^{99}\text{Tc}^{\text{m}}$ -activity of the blood in the erythrocyte fraction during the experimental period indicating stability of the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex. The labelling efficiency of $^{99}\text{Tc}^{\text{m}}$ -plasmin has previously been determined to be $91 \pm 2.5\%$ (Edenbrandt *et al.*, 1982) by scanning of a gel chromatography column (Persson & Darte, 1977).

It has been reported that scintillation camera images using the $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex may be of value in the diagnosis of DVT (Beswick *et al.*, 1979; Kempf & van der Linden, 1981). Our interpretation is that conventional scintillation camera images, produced after intravenous injection of $^{99}\text{Tc}^{\text{m}}$ -erythrocyte complex into a cubital vein, are not useful for the diagnosis of DVT in the calves. The technique seems, however, to have a certain but limited value for the diagnosis of DVT in popliteal and femoral veins. Thus, using this technique, a positive diagnosis of DVT, determined as defects in the vessels, could be made in these regions in four out of seven patients with no false positive findings.

Acknowledgments

This study has been supported by grants from Thorsten and Elsa Segerfalks Foundation for Medical Research, from Malmöhus Läns Landsting and from the Swedish National Association against Heart and Chest Diseases.

References

- ADOLFSSON L., NORDENFELT I., OLSSON H. & TORSTENSSON I. (1982) Diagnosis of deep vein thrombosis with $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta med scand*, **211**, 365–368.
- ARMITAGE P. (1980) *Statistical Methods in Medical Research*. Blackwell Scientific Publications, Oxford.
- BESWICK W., CHMIEL R., BOOTH R., VELLAR I., GILFORD E. & CHESTERMAN C. N. (1979) Detection of deep venous thrombosis by scanning of $^{99\text{m}}$ technetium-labelled red-cell venous pool. *Br Med J*, **i**, 82–84.
- EDENBRANDT C.-M., ANDERSSON L., LARSSON I., NILSSON J., OHLIN P., STRAND S.-E. & STÄHLBERG F. (1983) Diagnosis of deep venous thrombosis by $^{99\text{m}}$ Tc-human serum albumin microcolloid. *Eur J Nucl Med*, **8**, 332–334.
- EDENBRANDT C.-M., NILSSON J. & OHLIN P. (1982) Diagnosis of deep venous thrombosis by phlebography and $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta med scand*, **211**, 59–64.
- GULLMO Å. (1956) On the technique of phlebography of the lower limb. *Acta Radiologica*, **46**, 603–620.

- HAVIG Ö. (1977) Deep vein thrombosis and pulmonary embolism. *Acta chir scand*, (suppl.), 478.
- HEDNER U., JOHANSSON L. & NILSSON I. M. (1978) Effects of porcine plasmin on the coagulation and fibrinolytic systems in humans. *Blood*, **51**, 157–164.
- HLADIK W. B., NIGG K. K. & RHODES B. A. (1982) Drug-induced changes in the biologic distribution of radiopharmaceuticals. *Semin Nucl Med*, **2**, 184–218.
- HUBERTY I. P. & HATTNER R. S. (1982) A modified method for the in vivo labelling of red blood cells with Tc-99m. *J nucl Med*, **23**, 945–946.
- KEMPI V. & VAN DER LINDEN W. (1981) Diagnosis of deep vein thrombosis with in vivo $^{99}\text{Tc}^{\text{m}}$ -labelled red blood cells. *Eur J Nucl Med*, **6**, 5–9.
- KAHN O., ELL P. J., CULLUM I. D., JARRIT P. H. & WILLIAMS E. S. (1981) Clinical and pharmacological studies with $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Semin Nucl Med*, **2**, 157–171.
- MALAMUD H. (1978) Dosimetry of $^{99}\text{Tc}^{\text{m}}$ -labelled blood pool scanning agents. *Clin nucl Med*, **3**, 420–421.
- NICOLAIDES A. N. & OLSSON C. G. (1982) Applications of isotope technology to the clinical study of arterial and venous disease. In: *Noninvasive Techniques in Vascular Disease*. (ed. E. F. Bernstein), pp. 159–160. CV Mosby Company, London.
- OLSSON C. G., ALBRECHTSSON U., DARTE L. & PERSSON R. B. R. (1982) Tc-99m plasmin for rapid detection of deep vein thrombosis. *Nucl Med Commun*, **3**, 41–52.
- PERSSON R. B. R. & DARTE L. (1977) Labelling plasmin with technetium-99m for scintigraphic localization of thrombi. *Int J appl Radiat Isotopes*, **28**, 97–104.
- PERSSON B., OLSSON C. G., DARTE L., STRAND S. E., BERGQVIST L. & STÅHLBERG F. (1981) Preparation and testing of $^{99}\text{Tc}^{\text{m}}$ -labelled plasmin for thrombus detection. In: *Progress in Radiopharmacology*. (ed. P. H. Cox), vol. 2, pp. 147–156. Elsevier/North Holland Biomedical Press, Amsterdam.
- SMITH T. D. & RICHARDS P. (1976) A simple kit for the preparation of $^{99}\text{Tc}^{\text{m}}$ -labelled red blood cells. *J nucl Med*, **17**, 126–131.
- TENBORN L. & HEDNER U. (1981) Demonstration of $^{99}\text{Tc}^{\text{m}}$ -labelled plasmin and α_2 -antiplasmin on the surface of ex vivo thrombi. In: *Progress in Fibrinolysis*. (eds. J. F. Davidson, I. M. Nilsson & B. Åstedt), pp. 324–328. Churchill-Livingstone, Edinburgh.

CRITERIA FOR DETECTION OF DEEP VENOUS THROMBOSIS BY $^{99}\text{Tc}^{\text{m}}$ - PLASMIN TEST

C.M. Edenbrandt¹ and L. Brudin².

From the Departments of Medicine¹ and Clinical Physiology², Lasarettet,
Helsingborg, Sweden.

Correspondence to: Dr C. M. Edenbrandt

Present address: Department of Clinical Chemistry
University of Lund
Malmö General Hospital
S - 214 01 Malmö
Sweden

Summary

Criteria for the interpretation of ⁹⁹Tc^m-plasmin test results were systematically investigated in 353 patients with suspected deep venous thrombosis (DVT). Scintillation detector measurements were made at 12 points on each leg after intravenous injection of ⁹⁹Tc^m-porcine plasmin. Phlebography was used as reference method.

The criteria were chosen to obtain the highest possible sensitivity (98-100 %). On this assumption, the specificity reached 66 - 69 %, when using the two most efficient criteria. These criteria involved a comparison between count rates at three adjacent points of the leg suspected of having DVT, and count rates at the three corresponding points of the other leg. Comparison between measurements performed at 5 min and 30 min after the injection showed a higher sensitivity of the test at the 30 min measurement. The specificity decreased when the results from both 5 and 30 min measurements were combined. Further increase in specificity could only be achieved at the cost of decreased sensitivity. The suggested criteria seem to be the most efficient for this kind of radionuclide test and improved test-efficiency has to await more thrombus-specific radiopharmaceuticals.

Introduction

In the last few years several reports have documented a high sensitivity of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test in detecting deep venous thrombosis (DVT) in the legs (1-8). This radionuclide test is non-invasive, rapid, inexpensive and convenient to the patient. However, its clinical use has been hampered by the large number of falsely positive tests, i.e. a low specificity.

A criterion for determining whether a $^{99}\text{Tc}^{\text{m}}$ - plasmin test result is indicating DVT, i.e. positive, or excluding DVT, i.e. negative, was proposed by Olsson et al. (1). Alternative criteria for the evaluation of $^{99}\text{Tc}^{\text{m}}$ - plasmin test results have not hitherto been systematically studied.

The aim of the present study has been to evaluate various criteria for the interpretation of results of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test, in order to increase the low specificity of the test without reducing the high sensitivity previously attained using the conventional criterion (1).

Patients and methods

Consecutive patients admitted to the medical emergency ward due to suspected DVT were included in the study. Three groups of patients were studied.

Group A comprised 134 patients previously reported (4). Of these, 28 patients were excluded, 23 due to incomplete phlebography and 5 due to difficulties in the interpretation of the phlebogram, caused by extreme post-thrombotic sequelae. The remaining 106 patients, 50 men and 56 women, with a mean age of 60 (range 16 - 87) years were studied *retrospectively*.

Group B comprised 121 patients and were also studied *retrospectively*. Twelve patients were excluded due to incomplete phlebography. Thus, 109 patients, 51 men and 58 women, with a mean age of 61 (range 19 - 88)

⁴ Criteria for ⁹⁹Tc^m- plasmin test

years were included.

Group C comprised 157 patients of whom 19 patients were excluded, 15 due to incomplete phlebography, 3 due to difficulties in interpreting the phlebogram because of extreme post-thrombotic sequelae and one patient because the ⁹⁹Tc^m- plasmin test was performed after phlebography. The remaining 138 patients, 50 men and 88 women, with a mean age of 61 (range 22 - 95) years were studied *prospectively*.

⁹⁹Tc^m-plasmin test

The ⁹⁹Tc^m-plasmin test was performed as previously described (4). About 20 MBq of ⁹⁹Tc^m- porcine plasmin (LysofibrinTM, NOVO A/S, Denmark) was injected into an antecubital vein. The accumulation of radioactivity at each of twelve points from the ankle to the groin of each leg was measured by external counting using a collimated NaI(Tl)-detector (ThrombographTM, NOVO A/S, Denmark). Patients in groups A and B were measured at 5 min after the injection. If the test was considered positive at this stage using the conventional criterion, no more measurements were made. If the test was negative after 5 min, measurements were also made at 30 min after injection. This procedure is identical to that proposed by Olsson et al. (1). In patients of group C, the measurements were consistently made at both 5 and 30 min after the injection.

Phlebography

Phlebography was used as a control and was performed as previously described (9) following the ⁹⁹Tc^m-plasmin test. All patients in group A were examined. Patients in group B were examined only if the ⁹⁹Tc^m- plasmin test was positive according to the criterion previously proposed (1). Patients in group C were subjected to phlebography only if the ⁹⁹Tc^m-

plasmin test was positive at 5 and/or 30 min after the injection according to the conventional criterion (1) or according to criteria assessed in this study. Since it was not possible to examine all patients by phlebography and the proposed criterion (1) had previously shown no negative results in patients with DVT (4), we considered a negative test, according to this criterion (1) in group B and in all the criteria examined in group C, as equivalent to a negative phlebogram, which together with results of phlebography constituted the 'reference method'.

Criteria

The most commonly used criterion for the interpretation of $^{99}\text{Tc}^{\text{m}}$ - plasmin test results was proposed by Olsson et al.(1). This criterion is based on the difference between the count rates measured at a particular leg point (i) of the right and left legs. This difference is expressed in 'quotient units' (qu) and is defined as:

$$Q = \frac{C_{Ri}}{C_{Ri} + C_{Li}} \times 100 - 50$$

where

C_{Ri} = count rate at point number i (i=1 to 12) of the right leg

C_{Li} = count rate at the corresponding point of the left leg

The $^{99}\text{Tc}^{\text{m}}$ -plasmin test is considered positive if $|Q| \geq 13$ qu for any pair of points or if $|Q| \geq 3$ qu at each of three adjacent pairs of points, provided the excessive count rates are obtained over the suspected leg. In the subsequent evaluation these limits are called 'criterion I'.

The purpose of this study has been to evaluate new criteria in order to increase the specificity of the test without a consequent decrease in

sensitivity. Q was calculated for each pair of corresponding leg points (12 for each subject). The greatest of these values was called the one- pair-maximum or 1-Pmax. Values of Q in every two adjacent points were thereafter summed up and the greatest value of these 11 sums was called the two-pair-maximum or 2-Pmax. Subsequently 3-Pmax and 4-Pmax were calculated in the same way by summing up Q-values of 3 and 4 adjacent leg points, respectively.

The alternative criteria were assessed by using results obtained in patients from group A. First, 1-Pmax, 2-Pmax, 3-Pmax and 4-Pmax were calculated for each of the 106 patients. In order to maintain a high sensitivity, optimal levels of these values or any combination thereof were then assessed from the 43 patients with DVT according to phlebography. Then, in terms of specificity, the five most efficient criteria were chosen ('criteria II to VI', Table I) and subsequently evaluated in patients from groups A, B (retrospectively) and C (prospectively).

Statistical methods

The data were analysed according to McNemar's method and the test was carried out by using the exact binomial distribution (10).

Results

The criterion proposed by Olsson et al. (1) and the five alternative criteria (Table I) were first evaluated in a retrospective study (group A patients, Table II). Due to the selection of the new criteria an increase in specificity from 48 % to between 62 % and 64 % ($p < 0.01$) was achieved when using any of the alternative criteria compared to the conventional criterion (1), but at the cost of one falsely negative test. Although this patient had a thrombus from mid-calf to mid-thigh, a very low difference

in count rates was obtained between the two legs.

Secondly, the alternative criteria were evaluated retrospectively in patients of group B (Table III). According to the selection of patients who were subjected to phlebography, criterion I showed a 100% sensitivity. In comparison with this criterion the alternative criteria resulted in a slight reduction of falsely positive tests (from 30 to between 26-29 patients), at the cost of between two and three falsely negative tests (reduced sensitivity). Concerning these falsely negative tests, however, measurements were performed only at 5 min after the injection. In two of these patients phlebography showed a small thrombus in the fibular and tibial vein, respectively, and in one patient a thrombus extending from mid-calf to mid-thigh.

In group C, measurements were made at both 5 and 30 min after the injection. The results of the six criteria are shown in Table IV. The specificity was higher when using the alternative criteria compared to the conventional criterion, but in most cases at the cost of lower sensitivity. The specificity compared to criterion I was significantly higher ($p < 0.05$) for criteria II to VI, when combining the measurements at 5 and 30 min. All patients with falsely negative tests had DVT confined to the calf.

Considering criteria II - VI it was found that the number of falsely negative tests in criteria III (30 min) could be reduced from four to one by decreasing the limit of $|\Delta\text{Pmax}|$ from 14.3 qu to 13.9 qu. A similar reduction could not be obtained for the other alternative criteria without decreasing specificity. This modified criterion did not affect the sensitivity and the specificity in group A, B or C (5 min) but increased the sensitivity in group C (30 min) and group C (5+30 min) from four and two falsely negative tests, respectively, to only one. This patient had calf vein thrombosis at admission according to unilateral phlebography. Bilateral

phlebography performed ten days later due to deterioration in spite of anticoagulant therapy showed DVT in both legs. The falsely negative test at the admission might therefore have been due to bilateral thrombosis. Considering criterion I and the modified criterion III, measurements performed only 30 min after the injection showed no and one falsely negative test, respectively, compared to the four and three falsely negative tests, respectively, obtained 5 min after injection. When combining the measurements at 5 and 30 min, the sensitivity remained unchanged but the specificity decreased compared to the single 30 min measurement, for both criterion I ($p < 0.05$) and the modified criterion III.

Considering group B and C (5+30 min) together, we found that the number of falsely positive tests was reduced from 68 to 58 for criteria II and IV ($p < 0.05$) and from 68 to 59 for criterion VI ($p < 0.05$), compared to criterion I. The improved specificity was achieved at the cost of 5, 3 and 4 falsely negative tests, respectively, corresponding to a sensitivity of between 94 % and 96 %, but this was not statistically significantly different from 100 %.

Discussion

Radiopharmaceuticals so far used for detecting DVT are to a great extent reflecting the blood distribution in the leg (11). Because of this, there is a variation in count rates along the leg making it difficult to compare count rates obtained at different points along the same leg. This problem has previously been overcome by comparing count rates from corresponding points of the *two* legs, but at the cost of being less able to detect bilateral thrombosis (1, 12). Indeed, all criteria published so far, regarding the $^{99}\text{Tc}^{\text{m}}$ - plasmin test (1, 3, 5, 13), in addition to the criteria presented in this study, have utilized this principle.

In the search for alternative criteria our attention has been focused on criteria which are unbiased by subjective judgements. No attempt has therefore been made to interpret scintillation camera images as suggested by others (5, 7). Furthermore, since the $^{99}\text{Tc}^{\text{m}}$ - plasmin test is designed to be used as a screening test for patients with suspected DVT, a high sensitivity is required.

In the present study we have evaluated various criteria for the interpretation of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test using an established reference method, phlebography, and compared these criteria with the conventional one (1). The most efficient new criterion ($|3\text{-Pmax}| \geq 13.9$ qu) and criterion I both showed similar sensitivity (98% and 100 %) and specificity (69 % and 66 %), respectively, when measuring only 30 min after the injection. Further increase in specificity could only be achieved at the cost of decreased sensitivity. When using the alternative criteria in the prospective part of this study, falsely negative tests were found only in patients with calf vein thrombosis, but this was not considered acceptable since the clinical significance of small thrombi has recently been underlined (14).

When comparing results of the measurements performed at 5 and 30 min after the injection there was a higher sensitivity of the test at 30 min for both criterion I and the modified criterion III. Combining the results from the 5 min and 30 min measurements, as previously proposed (1), resulted in an unchanged sensitivity but a reduced specificity for both criteria, compared to the 30 min measurement.

Criteria II, IV and VI showed a statistically significant increase in specificity ($p < 0.05$) compared to the conventional criterion (1) when groups B and C (5+30 min) were combined. Nevertheless, the consequent decrease in sensitivity, 3-5 patients, was not considered satisfactory for

a screening test, even though this decrease in sensitivity was not statistically significant.

In conclusion, using criterion I from Olsson et al. (1) or the modified criterion III proposed in this study ($|3\text{-Pmax}| \geq 13.9$ qu) and with a single measurement at 30 min after injection, increased the specificity of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test from 59 % to 66 % and 69 %, respectively, compared to the conventional criteria, with a sensitivity still close to 100 %. A further increase in the specificity was not possible without a decrease in sensitivity. Thus, these two criteria seem to be equally valid and the most efficient found for this test and presumably for other radionuclide tests with the same mechanism of action (15). An improved test efficiency probably has to await more thrombus-specific radiopharmaceuticals.

Acknowledgements

This study has been supported by grants from Thorsten and Elsa Segerfalk's Foundation for Medical Research, Malmöhus Läns Landsting and the Swedish National Association against Heart and Chest Diseases.

References

1. Olsson CG, Albrechtsson U, Darte L, Persson RBR. $^{99}\text{Tc}^{\text{m}}$ -plasmin for rapid detection of deep vein thrombosis. Nucl Med Commun 1982;3:41-52.
2. Khan O, Ell PJ, Cullum ID, Jarrit PH, Williams ES. Clinical and pharmacological studies with $^{99}\text{Tc}^{\text{m}}$ -plasmin. In: Cox PH (ed) Progress in radiopharmacology. Amsterdam: Elsevier/North Holland Biomedical Press, 1981;157-171.

3. Adolfsson L, Nordenfelt I, Olsson H, Torstensson I. Diagnosis of deep vein thrombosis with $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta Med Scand* 1982; 211: 365-368.
4. Edenbrandt CM, Nilsson J, Ohlin P. Diagnosis of deep venous thrombosis by phlebography and $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta Med Scand* 1982;211:59-64
5. Dahlborn M, Ahlborg G, Söderborg B, Virgin J, Wilczec B. Gamma camera detection of $^{99}\text{Tc}^{\text{m}}$ -plasmin in the diagnosis of deep-vein thrombosis. *Eur J Nucl Med* 1984;9:499-501.
6. Husted SE, Kraemmer-Nielsen H, Krusell L, Fasting H, Østergaard-Nielsen B, Bruun-Pedersen J, Dalgaard E, Hvid-Hansen H. Deep vein thrombosis detection by $^{99}\text{Tc}^{\text{m}}$ -plasmin test and phlebography. *Brit J Surg* 1984;71:65-66.
7. Lückers AEG, Luth WJ, Teule GJJ, Huijgens PC, Heidendal GAK. Diagnosis of deep venous thrombosis with $^{99}\text{Tc}^{\text{m}}$ -plasmin using the gamma camera. *Eur J Nucl Med* 1984;9:282-283.
8. Aronen HJ, Korppi-Tommola T, Suoranta HT, Taavitsainen MJ. $^{99}\text{Tc}^{\text{m}}$ -plasmin test in deep vein thrombosis of the leg. *Eur J Nucl Med* 1985;10:10-12.
9. Gullmo Å. On the technique of phlebography of the lower limb. *Acta Radiologica* 1956;46: 603-620.

10. Fleiss JL. Statistical Methods for Rates and Proportions. John Wiley & Sons, Inc., New York 1981.
11. Edenbrandt CM, Dahlström JA, Nilsson J, Ohlin P. Comparison between $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin and $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes in diagnosis of deep vein thrombosis. Clinical Physiology 1984;4:243-252.
12. Browse NL, Clapman WF, Croft DN, Jones DJ, LeaThomas M, Olwen Williams J. Diagnosis of established deep vein thrombosis with the ^{125}I - fibrinogen uptake test. Br Med J 1971;4:325-328.
13. Deacon JM, Ell PJ, Anderson P, Khan O. Technetium $^{99\text{m}}$ -plasmin: a new test for the detection of deep vein thrombosis. Brit J Radiol 1980;53:673-677.
14. Havig Ö. Deep vein thrombosis and pulmonary embolism. Acta Chir Scand 1977; Suppl 478.
15. Ståhlberg F, Andersson L, Edenbrandt CM, Strand SE. Quantitative *in vivo* and *in vitro* comparison between radiolabelled colloids, biomolecules and blood cells in their ability to diagnose deep venous thrombosis. Nucl Med Commun 1984;5:741-762.

Table I. Criteria for the interpretation of $^{99}\text{Tc}^{\text{m}}$ -plasmin test results. The values are expressed in quotient units (qu). In addition to the limits below it is also assumed, for a positive test, that the excessive count rates are obtained over the suspected leg.

<u>Criteria</u>	<u>Limits for a positive test</u>
I	$ Q \geq 13$ qu in one pair of leg points or $ Q \geq 3$ qu in each of three adjacent pairs of leg points
II	$ 1\text{-Pmax} \geq 5.0$ qu and $ 2\text{-Pmax} \geq 9.8$ qu and $ 3\text{-Pmax} \geq 14.3$ qu and $ 4\text{-Pmax} \geq 15.3$ qu
III	$ 3\text{-Pmax} \geq 14.3$ qu
IV	$ 1\text{-Pmax} \geq 8.7$ qu and $ 4\text{-Pmax} \geq 18.2$ qu
V	$ 2\text{-Pmax} \geq 12.2$ qu and $ 4\text{-Pmax} \geq 18.2$ qu
VI	$ 3\text{-Pmax} \geq 15.5$ qu and $ 4\text{-Pmax} \geq 18.2$ qu

Table II. Comparison between phlebography and the $^{99}\text{Tc}^{\text{m}}$ -plasmin test for the diagnosis of DVT in 106 patients (group A). The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was interpreted according to the criterion (I) proposed by Olsson et al. (1) and five alternative criteria (II-VI). Sensitivities and specificities for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test are indicated.

		$^{99}\text{Tc}^{\text{m}}$ -plasmin test											
		Criteria											
PHLEBOGRAPHY	I		II		III		IV		V		VI		
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	
	43	0	42	1	42	1	42	1	42	1	42	1	
	33	30	24	39	24	39	23	40	23	40	24	39	
SENSITIVITY (%)	100		98		98		98		98		98		
SPECIFICITY (%)	48		62		62		64		64		62		

Table III. Comparison between the 'reference method' (see methods) and the $^{99}\text{Tc}^{\text{m}}$ -plasmin test for the diagnosis of DVT in 109 patients (group B). The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was interpreted according to the criterion (I) proposed by Olsson et al.(1) and five alternative criteria (II-VI). Values of the sensitivity and specificity for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test are indicated.

$^{99}\text{Tc}^{\text{m}}$ -plasmin test												
Criteria												
REFERENCE METHOD	I		II		III		IV		V		VI	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
	34	0	32	2	32	2	32	2	31	3	32	2
	30	45	27	48	29	46	26	49	29	46	28	47
SENSITIVITY (%)	100		94		94		94		91		94	
SPECIFICITY (%)	60		64		61		65		61		63	

Table IV. Comparison between the 'reference method' (see methods) and the $^{99}\text{Tc}^{\text{m}}$ -plasmin test for the diagnosis of DVT in 138 patients (group C). The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was performed at both 5 min (a) and 30 min (b) after the injection. The results were interpreted according to the conventional criterion (I) and five alternative criteria (II-VI). The results of the two measurements were also combined (a+b) and considered positive when the test was positive at any of the two measurements.

$^{99}\text{Tc}^{\text{m}}$ -plasmin test												
Criteria												
(a) REFERENCE METHOD	I		II		III		IV		V		VI	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
	41	4	41	4	42	3	42	3	42	3	41	4
	28	65	23	70	23	70	23	70	23	70	22	71
SENSITIVITY (%)		91	91		93		93		93		91	
SPECIFICITY (%)		70	75		75		75		75		76	
(b) REFERENCE METHOD	I		II		III		IV		V		VI	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
	45	0	41	4	41	4	42	3	42	3	42	3
	32	61	29	64	29	64	28	65	29	64	28	65
SENSITIVITY (%)		100	91		91		93		93		93	
SPECIFICITY (%)		66	69		69		70		69		70	
(a + b) REFERENCE METHOD	I		II		III		IV		V		VI	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
	45	0	42	3	43	2	44	1	44	1	43	2
	38	55	31	62	31	62	32	61	32	61	31	62
SENSITIVITY (%)		100	93		96		98		98		96	
SPECIFICITY (%)		59	67		67		66		66		67	

Return to normal of $^{99}\text{Tc}^{\text{m}}$ -plasmin test after deep venous thrombosis and its relation to vessel wall fibrinolysis.

C.M. Edenbrandt¹, U. Hedner², J. Nilsson³, P. Ohlin⁴ and L. Tengborn².

Departments of Medicine¹, Diagnostic Radiology³ and Clinical Physiology⁴, Lasarettet, Helsingborg, Sweden and Department of Coagulation Disorders², University of Lund, Malmö General Hospital, Malmö, Sweden.

Offprint requests to: Dr C. M. Edenbrandt.

Present address: Department of Clinical Chemistry
University of Lund
Malmö General Hospital
S - 214 01 Malmö
Sweden

Abstract. Fourteen patients with deep venous thrombosis (DVT) and positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test were followed in order to determine how soon a negative test is obtained. Localization and extension of the thrombi were determined by phlebography. The plasminogen activator activity in vein walls and the local fibrinolytic activity after venous occlusion were measured to disclose prerequisites of impaired thrombolysis.

The time required to obtain a negative $^{99}\text{Tc}^{\text{m}}$ -plasmin test showed a considerable variation, ranging from less than one week to more than six months. The $^{99}\text{Tc}^{\text{m}}$ -plasmin test had returned to normal in 64 % of the patients after 6 months. No relation was found between vessel wall fibrinolysis and time to normalization. Instead, we found an association between the time to normalization of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test and the size of the thrombus, according to phlebography, as well as between the time to normalization of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test and the extension of leg points with a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test at admission.

The finding of abnormal $^{99}\text{Tc}^{\text{m}}$ -plasmin test results more than six months after acute DVT is of practical importance and warrants caution when evaluating patients with symptoms and signs suggestive of acute recurrent DVT.

Key words: Deep venous thrombosis
 $^{99}\text{Tc}^{\text{m}}$ -plasmin test
 Plasminogen activator activity
 Fibrinolytic activity in vessel wall

Introduction

The diagnostic value of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test for detecting acute deep venous thrombosis (DVT) in the legs has been established (Khan et al., 1981; Adolfsson et al., 1982; Edenbrandt et al., 1982; Olsson et al., 1982; Dahlborn et al., 1984; Husted et al., 1984; Lückers et al., 1984; Aronen et al., 1985). The present investigation concerns how soon patients with DVT and a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test obtain a negative test result. It should be of practical importance to know when the $^{99}\text{Tc}^{\text{m}}$ -plasmin test can be reapplied to patients with renewed symptoms and signs compatible with acute rethrombosis. Regarding other investigative procedures, the course of normalization after DVT has been followed by phlebography (Bergvall & Hjelmstedt, 1968; Becker et al., 1970; Thomas & McAllister, 1971; May & Nissl, 1979) and impedance plethysmography (Jay et al., 1984) but hitherto very little is known about the course of normalization of radionuclide tests after DVT.

We also wanted to look into the question of what determines the normalization time. Both extension of the DVT and treatment have been reported to influence how soon the phlebographic result returns to normal (Young et al., 1974; May & Nissl, 1979; Arnesen et al., 1982). It has also been shown that patients with recurrent 'idiopathic' DVT often have impaired fibrinolytic activity in the vessel wall and/or in plasma (Isacsson & Nilsson, 1972), which could predispose to impaired thrombolysis and thus delayed normalization of test results. Indeed, Peduzzi et al. (1981) reported that patients with poor recanalization of retinal vein occlusion, as measured by dromofluorograms, had low fibrinolytic activity after venous occlusion. Particular interest was therefore paid to the question whether fibrinolytic activity in vein walls and/or in plasma after venous occlusion were related to the time period until the $^{99}\text{Tc}^{\text{m}}$ -plasmin test was normalized.

Patients and methods

Fourteen patients, seven women and seven men, with a mean age of 64 (range 28-82) years, admitted to the Department of Medicine due to DVT were included in the study. Seven out of the 14 patients had previously experienced thrombo-embolic disease (Table I). Pulmonary embolism (PE) had been verified in two patients by lung scintigraphy, 22 and 25 months earlier, respectively. DVT had been verified in three patients by phlebography, seven months, 21 months and six years earlier, respectively. Two patients had experienced DVT and PE, respectively, 14 and 23 years ago, according to clinical diagnosis.

Informed consent to repeated investigations was obtained from each patient and the study was approved by the Human Ethics Committee, Faculty of Medicine, University of Lund.

On admission day the $^{99}\text{Tc}^{\text{m}}$ -plasmin test was positive in all patients and the extent and localization of the DVT was shown by phlebography. Symptoms, signs and any history of thrombo-embolic disease were registered. All patients received heparin intravenously for the first five to seven days, during which time oral dicoumarol therapy was started. The median duration of oral anticoagulant therapy was 4.3 months (range 2 - 12 months). The effectiveness of the anticoagulant treatment in all patients was such that 86 % of the test results were between five and 20 percent ThrombotestTM (Nyegaard & Co., A/S, Norway) during the course of therapy.

The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was repeated at fixed intervals until it was normalized, i.e. at one week, six weeks, six months and twelve months after admission. Studies of the fibrinolytic system were performed in 10 patients 3 - 24 (median 14) months after admission, in order to exclude any influence of acute-phase reaction on the results. Three patients (A, J, and K) were on dicoumarol treatment at the time of investigation.

$^{99}\text{Tc}^m$ -plasmin test

The $^{99}\text{Tc}^m$ -plasmin test was performed in a standardized way as described earlier (Edenbrandt et al., 1982). About 20 MBq of $^{99}\text{Tc}^m$ - porcine plasmin (LysofibrinTM, NOVO A/S, Denmark) was injected into an antecubital vein . The accumulation of radioactivity at twelve points of each leg was measured by external counting using a collimated NaI(Tl) - detector (ThrombographTM, NOVO A/S, Denmark). The criteria for positive and negative tests were adopted from Olsson et al. (1982).

Phlebography

Phlebography was performed as described by Gullmo (1956). All phlebograms were re-evaluated by J.N., still unaware of the outcome of the other tests.

Fibrinolysis studies

Previously described methods were used for the following determinations: euglobulin clot lysis time, fibrinogen, plasminogen, degradation products of fibrinogen and fibrin, inhibitors of plasminogen activation by urokinase ('urokinase inhibitors') (Nilsson, 1974).

The plasminogen activator activity in vein walls (biopsy specimens from superficial hand veins) was determined according to Pandolfi's modification of Todd's fibrinolysis autography technique. The activity is expressed in arbitrary units (median value in 60 biopsy specimens of hand veins from healthy volunteers was 7.5 arbitrary units, range 6 - 10) (Nilsson & Pandolfi, 1976).

The local fibrinolytic activity before and after 20 min venous occlusion of both arms was measured on human fibrin plates known to be rich in plasminogen (Nilsson & Olow, 1962). Resuspended euglobulin precipitate was analysed. The determinations were performed in duplicate on two

consecutive days. The normal range ($250 - 550 \text{ mm}^2$) was defined as the 90 % confidence interval in a group of 64 apparently healthy individuals (Tengborn, Hedner & Nilsson, 1982).

Statistical methods

The data were analysed using Fisher's Exact Probability Test for Fourfold Tables (Armitage, 1980). A difference was considered significant when $p < 0.05$ (two-tailed).

Results

Phlebography

The localization and extension of the thrombi, as judged from the phlebograms, appear in Figure 1. Two patients (patients A and F) had two thrombi in the diseased leg.

$^{99}\text{Tc}^m$ - plasmin test

The $^{99}\text{Tc}^m$ -plasmin test was normalized in five patients already one week after admission (Table I). Five patients still had a positive $^{99}\text{Tc}^m$ -plasmin test when re-examined six months after admission. At the twelve month check-up the $^{99}\text{Tc}^m$ -plasmin test was negative in all patients except one (patient N), who complained about renewed symptoms during the preceding week from the same leg where he had had DVT twelve months earlier. The $^{99}\text{Tc}^m$ -plasmin test was more positive than it had been six weeks and six months after admission. A new phlebography showed rethrombosis. Figure 2 shows an example of the gradual normalization of repeated $^{99}\text{Tc}^m$ -plasmin tests. Patients with negative $^{99}\text{Tc}^m$ -plasmin test within one week (patients A-E) had more often a thrombus extension of less than seven leg points ($p < 0.05$) compared with patients (F-N) who had achieved normal $^{99}\text{Tc}^m$ - plasmin test after more than one week. Patients with

negative $^{99}\text{Tc}^{\text{m}}$ - plasmin test within six weeks (patients A-H) had more often a thrombus extension of less than eight leg points ($p < 0.05$) compared with patients (I-N) who had achieved normal $^{99}\text{Tc}^{\text{m}}$ -plasmin test after more than six weeks. Finally, patients with negative $^{99}\text{Tc}^{\text{m}}$ - plasmin test within six months (patients A-I) had more often a thrombus extension of less than ten leg points ($p < 0.05$) compared to patients (J-N) who had achieved normal $^{99}\text{Tc}^{\text{m}}$ -plasmin test after more than six months. The number and localization of leg points with positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test results in each patient at admission appear in Figure 1. The proportion of patients with more than six positive leg points was consistently greater with increasing normalization time: beyond one week ($p < 0.05$), beyond six weeks ($p < 0.01$), and beyond six months ($p < 0.05$). We also found that patients with normalized $^{99}\text{Tc}^{\text{m}}$ -plasmin test within six months had an extension of less than eight leg points with positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test more often than patients with normalized $^{99}\text{Tc}^{\text{m}}$ -plasmin test after six months ($p < 0.01$).

Clinical data

Symptoms and signs in each patient at admission were also evaluated (data not shown), but no statistically significant association was found with the time for normalization of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test. We also found no statistically significant difference in duration or effectiveness of treatment between the patient groups with regard to time to normalization of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test.

Fibrinolysis studies

All patients had normal values for fibrinogen (2.4 - 4.9 g/L), plasminogen (98 - 125 %), euglobulin clot lysis time (90 - 330 min), degradation products of fibrinogen and/or fibrin (0 - 7 mg/L) and inhibitors of

plasminogen activation by urokinase (81 - 131 %). No evidence of impaired vessel wall fibrinolysis was found (Table II).

Discussion

The time required for normalization of a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test in patients with DVT showed a considerable variation, ranging from less than one week to more than six months. The fact that the radionuclide test can remain positive for more than six months must be taken into account when examining patients who have had a recent history of DVT and then return with renewed symptoms and signs suggestive of recurrent DVT.

By measuring the distribution of $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes in the legs we recently showed (Edenbrandt et al., 1984) that there are marked circulatory changes in the leg with DVT compared to the other leg. We also found a similar distribution of $^{99}\text{Tc}^{\text{m}}$ -plasmin as for $^{99}\text{Tc}^{\text{m}}$ -erythrocytes between the legs and concluded that the $^{99}\text{Tc}^{\text{m}}$ -plasmin was not bound to the thrombus to a significant extent but rather indirectly indicated the presence of DVT by reflecting the increased venous stasis or redistribution of blood flow secondary to the thrombus. Thus, repeating the $^{99}\text{Tc}^{\text{m}}$ -plasmin test, as performed in the present study, gave us a convenient probe to follow recovery of circulatory changes secondary to DVT. We then found that patients with extended thrombi not only had the most pronounced circulatory changes in the acute phase but also required a longer time to normalize their functional changes. It is interesting to notice the similarities between the results obtained by Jay et al. (1984) for plethysmography and the results of the present study utilizing the $^{99}\text{Tc}^{\text{m}}$ - plasmin test. In their study the plethysmographic results had returned to normal in 48 % of the patients after 6 weeks and in 60 % of the patients after 3 months, compared to 57 % of the patients after 6 weeks and 64 % of the patients after 6 months, in our study. This is in contrast to

phlebography where May & Nissl (1979) reported that recanalization is rarely seen before four months in patients given only anticoagulant therapy. However, the normalization of the $^{99}\text{Tc}^{\text{m}}$ -plasmin test does not necessarily indicate significant lysis of the thrombus but may merely reflect the development of a generous collateral circulation which was also shown to occur past the obstructing thrombus during the recovery period (May & Nissl, 1979).

Abnormal fibrinolytic activity was not found in the present patients with DVT. Even those patients with a previous history of thromboembolic disease (7/14) showed normal fibrinolytic activity. Furthermore, the results of the present study did not indicate any relationship between time to normalization of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test and fibrinolytic activity. Neither could the variation in normalization time of the radionuclide test be explained by differences in intensity or duration of anticoagulant therapy. So, the present study substantiates the relative independence of normalization of the radionuclide test and fibrinolytic activity.

Acknowledgements

This study has been supported by grants from Thorsten and Elsa Segerfalk's Foundation for Medical Research, Malmöhus Läns Landsting and the Swedish National Association against Heart and Chest Diseases.

References

- Adolfsson L, Nordenfelt I, Olsson H, Torstensson I (1982) Diagnosis of deep vein thrombosis with $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta Med Scand* 211: 365 - 368
- Armitage P (1980) *Statistical Methods in Medical Research*. Blackwell Scientific Publications, Oxford
- Arnesen H, Hoiseth A, Ly B (1982) Streptokinase or heparin in the treatment of deep vein thrombosis. Follow-up results of a prospective study. *Acta Med Scand* 211: 65 - 68
- Aronen HJ, Korppi-Tommola T, Suoranta HT, Taavitsainen MJ (1985) $^{99}\text{Tc}^{\text{m}}$ - plasmin test in deep vein thrombosis of the leg. *Eur J Nucl Med* 10: 10 - 12
- Becker J, Borgström S, Saltzman G-F (1970) Occurrence and course of thrombosis following prostatectomy. *Acta Radiol Diagnosis* 10: 513 - 533
- Bergvall U, Hjelmstedt Å (1968) Recanalization of deep venous thrombosis of the lower leg and thigh. *Acta Chir Scand* 134: 219 - 228
- Dahlborn M, Ahlborg G, Söderborg B, Virgin J, Wilczec B (1984) Gamma camera detection of $^{99}\text{Tc}^{\text{m}}$ -plasmin in the diagnosis of deep-vein thrombosis. *Eur J Nucl Med* 9: 499 - 501
- Edenbrandt CM, Nilsson J, Ohlin P (1982) Diagnosis of deep venous thrombosis by phlebography and $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta Med Scand* 211: 59 - 64
- Edenbrandt CM, Dahlström JA, Nilsson J, Ohlin P (1984) Comparison between $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin and $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes in diagnosis of deep vein thrombosis. *Clin Physiol* 4: 243 - 252
- Gullmo Å (1956) On the technique of phlebography of the lower limb. *Acta Radiologica* 46: 603 - 620
- Husted SE, Kraemmer-Nielsen H, Krusell L, Fasting H, Østergaard - Nielsen B, Bruun-Pedersen J, Dalgaard E, Hvid-Hansen H (1984) Deep vein thrombosis detection by $^{99}\text{Tc}^{\text{m}}$ plasmin test and phlebography. *Brit J Surg* 71: 65 - 66

Isacsson S, Nilsson IM (1972) Defective fibrinolysis in blood and vessel walls in recurrent 'idiopathic' venous thrombosis. *Acta Chir Scand* 138 : 313 - 319

Jay R, Hull R, Carter C, Ockelford P, Buller H, Turpie AGG, Hirsh J (1984) Outcome of abnormal impedance plethysmography results in patients with proximal-vein thrombosis: frequency of return to normal. *Thromb Res* 36: 259 - 263

Khan O, Ell PJ, Cullum ID, Jarrit PH, Williams ES (1981) Clinical and pharmacological studies with $^{99}\text{Tc}^{\text{m}}$ -plasmin. In: *Progress in radiopharmacology*. Cox PH (ed), Elsevier/North Holland Biomedical Press, Amsterdam, pp 157 - 171

Lückers AEG, Luth WJ, Teule GJJ, Huijgens PC, Heidendal GAK (1984) Diagnosis of deep venous thrombosis with $^{99}\text{Tc}^{\text{m}}$ -plasmin using the gamma camera. *Eur J Nucl Med* 9: 282 - 283

May R, Nissl R (1979) *Die Phlebographie der unteren Extremität*. George Thieme Verlag, Stuttgart, FRG

Nilsson IM, Olow B (1962) Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 123: 247 - 266

Nilsson IM (1974) *Haemorrhagic and Thrombotic Diseases*. J Wiley & Sons Ltd, London

Nilsson IM, Pandolfi M (1976) Assay of fibrinolytic activity of the vessel wall. In: Davidsson JF, Samama MM, Desnoyes PC (eds) *Progress in chemical fibrinolysis and thrombolysis*. pp. 1 - 13

Olsson CG, Albrechtsson U, Darte L, Persson RBR (1982) $^{99}\text{Tc}^{\text{m}}$ -plasmin for rapid detection of deep vein thrombosis. *Nucl Med Commun* 3: 41 - 52

Peduzzi M, DeRosa V, Fonda S, Coccheri S (1981) Haemostatic studies in retinal vein occlusion. Fibrinolytic response to venostasis as a prognostic factor for spontaneous recanalization. *Thromb Res* 24: 105 - 118

Tengborn L, Hedner U, Nilsson IM (1982) Prospective study of a Phenformine-like substance (moroxydine chloride) in patients with deficient vessel wall fibrinolysis. *J Med* 13: 353 - 364

Thomas ML, McAllister V (1971) The radiological progression of deep venous thrombus. *Radiology* 99: 37 - 40

Young AE, Thomas ML, Browse NL (1974) Comparison between sequelae of surgical and medical treatment of venous thromboembolism. *Brit Med J* 4: 127 - 130

Table I. Previous thromboembolic disease and time to normalization of positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test in 14 patients with DVT. *) Patient, who had re-thrombosis at 12 months check-up.

Patients	Previous DVT/PE	Negative $^{99}\text{Tc}^{\text{m}}$ -plasmin test (weeks after admission)
A	0	1
B	DVT	1
C	DVT	1
D	PE	1
E	0	1
F	0	6
G	DVT	6
H	DVT	6
I	0	26
J	PE	52
K	PE	52
L	0	52
M	0	52
N	0	*

Table II. Results of fibrinolysis studies in 10 patients with DVT. Patient identification as in Table I.

	Plasminogen activator content in vessel wall (arbitrary units)	Fibrinolytic activity of resuspended euglobulin fraction before and after venous occlusion (mm ²)	
Normal range	6 - 10	44-152	250-550
Patients			
A	9.0	232	439
B	7.0	120	633
F	9.0	210	474
G	6.5	143	631
I	6.0	110	402
J	8.0	181	453
K	7.5	212	477
L	6.5	160	468
M	6.0	143	638
N	9.0	214	620

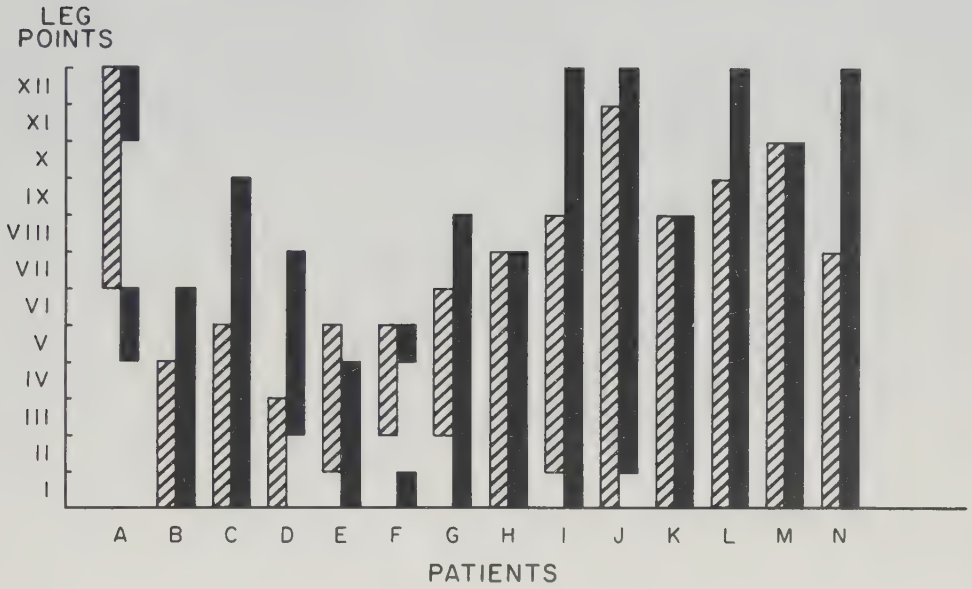


Figure 1. Leg points with positive $^{99}\text{Tc}^{\text{m}}$ - plasmin test (▨) and localization of DVT according to phlebography (■) in 14 patients at admission.

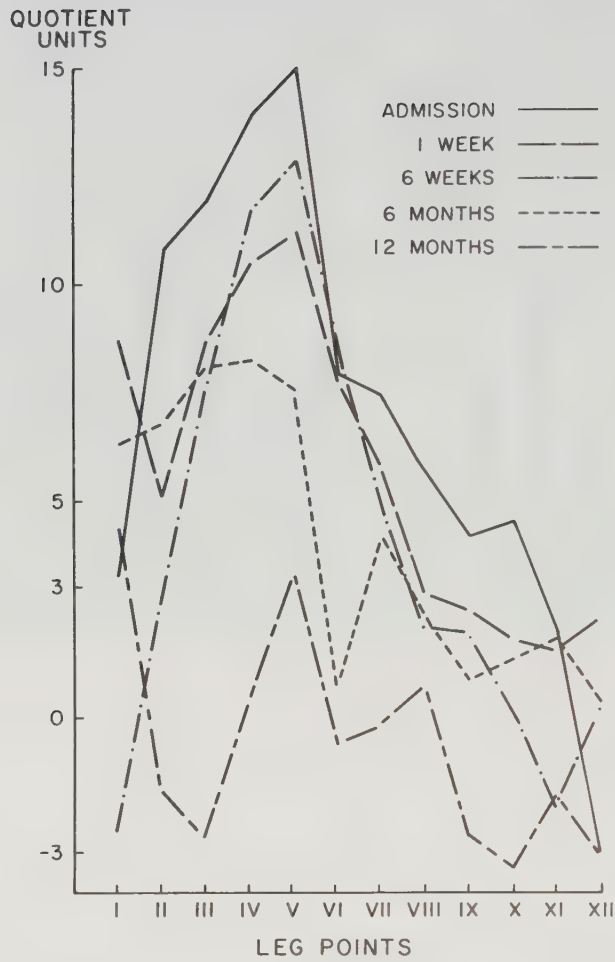


Figure 2. The relative $^{99}\text{Tc}^{\text{m}}$ -plasmin uptake in the diseased right leg of patient M, expressed in quotient units according to Olsson et al. (1982), at admission and after one week, 6 weeks, 6 months and 12 months. Phlebography showed a thrombus from leg point I to leg point X, at admission.

FOLLOW - UP OF CIRCULATORY CHANGES SECONDARY TO DEEP VENOUS
THROMBOSIS WITH SPECIAL REGARDS TO RADIONUCLIDE TESTS.

C.M. Edenbrandt¹, J. Nilsson² and P. Ohlin³.

From the Departments of Medicine¹, Diagnostic Radiology² and Clinical
Physiology³, Lasarettet, S- 251 87 Helsingborg, Sweden.

Correspondence to: Dr C.M. Edenbrandt

Present address: Department of Clinical Chemistry
University of Lund
Malmö General Hospital
S - 214 01 Malmö
Sweden

Summary

The influence of circulatory changes secondary to deep venous thrombosis (DVT) in the leg on results of radionuclide tests was studied in eight patients. Strain gauge plethysmography, a radionuclide blood pool test and phlebography were performed both in the acute phase and during recovery up to six months after initial admission. Morphological and functional changes were correlated to results from repeatedly performed $^{99}\text{Tc}^{\text{m}}$ -plasmin tests, a test currently used for diagnosis of DVT.

In the acute phase, the thrombotic leg showed increase of pooled blood and in the case of proximal thrombosis also impaired venous outflow. During the six month follow-up complete recanalization was found in three patients and partial recanalization in five. The circulatory changes were found to recover progressively and earlier than the morphological changes.

The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was pathological at admission in all patients. It was normalized in parallel with plethysmography and blood pool test results, at a time when morphological recovery was still incomplete. These findings corroborate that a positive $^{99}\text{Tc}^{\text{m}}$ - plasmin test reflects hemodynamic changes secondary to the DVT rather than a specific binding of the radiopharmaceutical to the thrombus. The $^{99}\text{Tc}^{\text{m}}$ -plasmin test was normalized from one to more than 26 weeks after an acute DVT. This finding is of practical importance when using radionuclide tests for evaluation of acute recurrent DVT.

Introduction

The importance of diagnosing DVT in the legs of symptomatic patients is well recognized (Havig, 1977; Hull and Hirsh, 1982). Olsson, Albrechtsson, Darte and Persson (1982) have shown the clinical usefulness of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test, its high sensitivity for DVT and its main disadvantage, its high number of false positive results. These findings have been confirmed by us (Edenbrandt, Nilsson and Ohlin, 1982) and others (Adolfsson, Nordenfelt, Olsson and Torstensson, 1982). However, there is still a controversy regarding the mechanism of action of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test. A specific interaction between $^{99}\text{Tc}^{\text{m}}$ - plasmin and the thrombus has been suggested as the cause of increased radioactivity in the thrombotic leg compared to the healthy leg (Khan, Ell, Cullum, Jarrit and Williams, 1981; Nicolaidis and Olsson, 1982; Gram and Jespersen, 1984; Dahlborn, Ahlborg, Söderborg, Virgin and Wilczek, 1984). We have recently shown that $^{99}\text{Tc}^{\text{m}}$ - plasmin has similar distribution as $^{99}\text{Tc}^{\text{m}}$ - labeled erythrocytes in both thrombotic and non-thrombotic legs, which implies circulatory changes secondary to the thrombus to account for the clinical usefulness of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test (Edenbrandt, Dahlström, Nilsson and Ohlin, 1984).

The present study was designed to further elucidate the mechanism of action of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test. Morphological and circulatory changes in the venous system of a leg with DVT was determined both in the acute phase and during six months follow-up. Methods used included phlebography, strain gauge plethysmography and a radionuclide blood pool test. The results of the three investigating procedures were compared with results of repeated $^{99}\text{Tc}^{\text{m}}$ - plasmin tests in the same patients.

Materials and methods

Patients

Eight patients, five men and three women, with a mean age of 64 (range 50-83) years, admitted to the Department of Medicine due to DVT were included in the study. At admission the patients were clinically examined and $^{99}\text{Tc}^{\text{m}}$ - plasmin test, a radionuclide blood pool test and phlebography were performed in that order on the same day. The results on admission for seven of these patients have previously been reported (Edenbrandt et al., 1984). The diagnosis was DVT in all cases according to phlebography. The patients were subsequently treated with heparin intravenously for five to seven days followed by dicoumarol therapy for 15 (range 10 - 26) weeks. One and six weeks after admission the $^{99}\text{Tc}^{\text{m}}$ - plasmin test was repeated and then also strain gauge plethysmography was performed. Six months after admission all patients were examined during two days. Clinical evaluation, strain gauge plethysmography and the blood pool test were performed on the first day. The $^{99}\text{Tc}^{\text{m}}$ - plasmin test and phlebography were performed on the second day.

This study was approved by the Human Ethics Committee, Faculty of Medicine, University of Lund. All patients gave their written consent to repeated investigations.

Clinical examination

At admission and at the six months check-up symptoms were asked for and signs including pain, swelling of calf (≥ 1 cm compared to the other leg), increase of temperature, erythema and dilatation of veins were looked for.

Phlebography

Phlebography was performed at admission and at six months follow-up as described by Gullmo (1956). All phlebograms were re-evaluated at the

same time by J.N., still unaware of the results of the other tests.

$^{99}\text{Tc}^{\text{m}}$ - plasmin test

The $^{99}\text{Tc}^{\text{m}}$ - plasmin test was performed in a standardized way as previously described (Edenbrandt et al., 1984). The criteria for positive and negative test results were adopted from Olsson et al. (1982). These criteria imply calculations of the quotient (Q) between count rates determined at a leg point of the diseased leg and the corresponding leg point of the other leg, expressed in 'quotient units'. In order to quantify the total results of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test, the twelve quotients (Q) calculated from each pair of corresponding leg points were summed up (Qsum). This sum of quotients was regarded as the total predominance of radioactivity in the diseased leg compared with the other leg.

Blood pool test

Autologous erythrocytes were labeled in vitro using $^{99}\text{Tc}^{\text{m}}$ - pertechnetate and after injection, scintillation detector measurements and scintillation camera images were performed as described previously (Edenbrandt et al., 1984). All images were evaluated by P.O. unaware of the outcome of the phlebography. The total predominance of radioactivity in the diseased leg compared with the other leg (Qsum) was calculated as for the $^{99}\text{Tc}^{\text{m}}$ - plasmin test. The in vivo stability of the $^{99}\text{Tc}^{\text{m}}$ - erythrocyte complex was analysed as described previously (Edenbrandt et al., 1984).

Strain gauge plethysmography

The plethysmographic examinations were performed according to Hallböök and Göthlin (1971). Mercury-in-silastic strain gauges (Medimatic, Denmark) were used and the recordings were made with a plethysmograph (SP2™, Medimatic, Denmark). Calf volume expansion (CVE) in response to a

congesting pressure of 50 mmHg and subsequent maximum venous outflow (MVO) were determined. At each examination (1 week, 6 weeks and 6 months) three measurements were performed and the mean used. In accordance with Hallböök and Göthlin (1971) a CVE of less than 2.2 ml/100 ml calf and a MVO of less than 40 ml/min/100 ml were considered pathological.

Statistical methods

The data were analysed using paired t-test and F-test after linear regression (Armitage, 1980). A difference was considered significant when $p \leq 0.05$. Data are given as median and range unless otherwise stated.

Results

Clinical examination

Clinical examination at admission revealed in each patient a median of eight (range five to ten) symptoms and signs. There was no association between the number of findings and the extent of DVT as shown by phlebography. At the six months check-up, seven patients were completely free of symptoms and signs in their legs. One patient with a ten year history of vasculitis complained about renewed symptoms during the preceding week. At the examination pain and tenderness were found to be localized superficially, more suggestive of vasculitis than of DVT.

Phlebography

Phlebography at admission showed distal thrombosis in three patients, i.e. DVT confined below the fissure of the knee, and proximal thrombosis in five patients, i.e. DVT confined above the knee or extending upwards from the calf. Marked dilatation of superficial vessels were found in three patients with proximal DVT and in one patient with distal DVT.

At the six months check-up complete recanalization was found in two of the distal DVT patients and partial recanalization in one. In patients with proximal thrombosis complete recanalization was found in one and partial recanalization in four. Patients with partial recanalization still had occluded parts of the veins. Post-thrombotic conditions, such as absence of valves and irregularities of the vessel lumen were found in all patients. The four patients with dilated superficial veins at admission showed the same findings at the six months check-up. No association was found between duration of anticoagulant therapy and extent of recanalization according to phlebography.

$^{99}\text{Tc}^m$ - plasmin test

The $^{99}\text{Tc}^m$ - plasmin test was positive in all patients at admission. The test was normalized one week later in two out of three patients with distal DVT but in none of the patients with proximal DVT. Two patients with proximal DVT showed normal $^{99}\text{Tc}^m$ - plasmin test at the six weeks check-up. Six months after admission only one patient with proximal DVT still had a positive $^{99}\text{Tc}^m$ - plasmin test (Table I). The total predominance of radioactivity in the diseased leg, calculated from the twelve corresponding pairs of leg points (Q_{sum}), decreased significantly ($p < 0.01$) from admission to the six months check-up in all patients.

Blood pool test

The labeling efficiency of the $^{99}\text{Tc}^m$ - erythrocytes, as measured in vitro, was 98.6 (73.8 - 99.3) % at admission and 98.4 (65.0 - 99.0) % at the six months check-up. The stability of the $^{99}\text{Tc}^m$ - erythrocyte complex was monitored during the procedure and was found as satisfactory as in a previous study (Edenbrandt et al., 1984).

The results of the scintillation detector measurements were

calculated and quantitated in a similar way as for the $^{99}\text{Tc}^{\text{m}}$ -plasmin test. Considering the total predominance of radioactivity in the diseased leg (Qsum) no significant difference was found at admission between the blood pool test and the $^{99}\text{Tc}^{\text{m}}$ - plasmin test. On the contrary, a close relationship ($p < 0.01$) was found between the results of the two tests (Fig. 1). The Qsum, calculated for the blood pool test, was also found to decrease significantly ($p < 0.01$) during the six months follow-up.

Considering scintillation camera images of the suspected leg at admission, well defined defects were found of the femoral and popliteal veins in four out of five patients with proximal DVT. One patient with proximal DVT and extensive collateral vessels according to phlebography showed tortuous and varicose vessels in the images to such an extent that the images could not be evaluated with certainty. Two out of three patients with distal DVT showed no defects or collaterals in their images. The third patient showed stenosis of the popliteal vein corresponding to the proximal part of the distal DVT. Marked collateral vessels were found in four out of five patients with proximal DVT and in one patient with distal DVT extending to the knee joint.

At the six months check-up, no defects were found in two patients with proximal DVT, both of whom had shown partial recanalization according to phlebography. Two patients with proximal DVT still showed defects of the main vessels in their images one of whom had shown complete recanalization according to phlebography. The images of one patient were still impossible to interpretate. All three patients with distal DVT showed no defects at the follow-up. In one patient with proximal DVT and in one patient with distal DVT the marked collateral vessels had disappeared at the six months check-up. In the other three patients with proximal DVT the collaterals still remained.

Strain gauge plethysmography

The first plethysmographic examinations were performed one week after admission. The three patients with distal DVT had normal MVO and CVE in both legs. Four out of five patients with proximal DVT had pathological MVO and CVE of the diseased leg (see Table I). The patient with MVO > 40ml/min/100 ml showed a biphasic emptying curve (Zetterqvist, Ericsson and Volpe, 1975) and phlebography showed a thrombus from the inguinal ligament to the most distal part of the caval vein. The patient with normal CVE showed extensive varicose veins of the calf, according to phlebography. Considering the healthy leg, all five patients with proximal DVT had normal CVE and three had normal MVO.

At the six weeks check-up two patients with proximal DVT obtained normal MVO and CVE of the diseased leg. After six months, CVE had been normalized in all patients but MVO was still pathological in two, both with partial recanalization according to phlebography. Considering the healthy leg, the two patients with pathological MVO at admission, still showed the same finding at the six months check-up.

The time for normalization of plethysmography results was generally in good agreement with the normalization time of the $^{99}\text{Tc}^{\text{m}}$ - plasmin test (Table I). However, one patient with distal DVT (below the popliteal vein) had normal MVO/CVE at one week after admission but did not have a normal $^{99}\text{Tc}^{\text{m}}$ - plasmin test until six months after admission. Phlebography showed partial recanalization with parts of the veins still occluded and marked dilatation of superficial vessels in the calf. Comparison between the time for normalization of $^{99}\text{Tc}^{\text{m}}$ - plasmin test results and plethysmography on one hand and findings at phlebography on the other revealed earlier normalization of the non-invasive tests than of the morphological changes.

Discussion

Our findings on admission day demonstrated that both distal and proximal DVT markedly increased count rates over the diseased leg, as measured by scintillation detector after i.v. injection of $^{99}\text{Tc}^{\text{m}}$ -labeled erythrocytes (Fig. 1). This finding indicates either an increased pooling of blood in the thrombotic leg or a more superficial localization of the blood flow with a decreased blood-detector distance. We also found a close relationship between results of the $^{99}\text{Tc}^{\text{m}}$ -plasmin tests and of the blood pool tests (Fig. 1). The results were similar to those obtained in a previous and more extensive study (Edenbrandt et al., 1984) and are in support of the hypothesis that a positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test reflects circulatory changes secondary to DVT.

Hemodynamic changes secondary to DVT have been evaluated by estimation of calf volume expansion (CVE) and maximum venous outflow (MVO) in response to a standardized congestive pressure (Dahn and Eiriksson, 1968; Hallböök and Göthlin, 1971; Sumner, 1981). We found positive $^{99}\text{Tc}^{\text{m}}$ -plasmin test and marked circulatory changes as measured by plethysmography in all patients with proximal DVT still one week after admission. In three patients with distal DVT the plethysmography was normal in all while the $^{99}\text{Tc}^{\text{m}}$ -plasmin test still was positive in one patient. The latter patient had varicose vessels according to phlebography which could explain the discrepancy. Repeated plethysmographic examinations showed a successive normalization of functional changes in patients with proximal DVT, which is well in accordance with previous studies (Dahn and Eiriksson, 1968; Hallböök and Göthlin, 1971; Ahlbäck et al., 1977; Jay, Hull, Carter, Ockelford, Buller, Turpie and Hirsh, 1984). During the follow-up period, the $^{99}\text{Tc}^{\text{m}}$ -plasmin test was normalized in parallel with plethysmographic results.

At the six months check-up, the blood pool test showed no major

difference in count rates between corresponding leg points of the thrombotic leg and the other leg (Fig. 1). This decreased pooling of blood or improved deep vein flow corresponded well to the improved plethysmographic and $^{99}\text{Tc}^{\text{m}}$ - plasmin test results.

The morphological basis to the improved functional changes was studied by repeat phlebography six months after admission. We found recanalization to occur in all patients and to be most farreaching in patients with small thrombi. However, *restitutio ad integrum* was never found and signs of post-thrombotic conditions remained in all patients in accordance with previous findings (May and Nissl, 1979). There are very few well-designed studies on the fate of a vein containing a thrombus. However, most authors agree that recanalization can be seen within days in patients on fibrinolytic therapy, within weeks to months in patients on heparin-dicoumarol treatment and after months to years in patients without therapy (Bergvall and Hjelmstedt, 1968; Becker, Borgström and Saltzman, 1970; Thomas and McAllister, 1971; Common, Seaman, Rosch, Porter and Dotter, 1976; Arnesen, Hoiseth and Ly, 1982; Rabinov and Paulin, 1983). In addition, small and distal thrombi have more often disappeared on repeat phlebograms than femoral and iliac thrombi (Young, Thomas and Browse, 1974; May and Nissl, 1979). Our phlebographic data are in agreement with these reports. More interesting is the finding in the present study that the functional changes, as measured by strain gauge plethysmography and the blood pool test, recovered earlier than the morphological changes. The same was true for symptoms and signs and the $^{99}\text{Tc}^{\text{m}}$ - plasmin test.

The similar behavior of the three non-invasive tests, both in the acute phase and during six months follow-up, corroborate that a positive $^{99}\text{Tc}^{\text{m}}$ - plasmin test reflects hemodynamic changes secondary to the DVT and not a specific binding of the radiopharmaceutical to the thrombus. This

concept facilitates understanding of the advantages and limitations of the radionuclide test. In established DVT, hemodynamic changes are already present at admission of the patient and thus can be measured instantly making up for a rapid test result. However, this type of functional changes are not specific for acute DVT which presumably accounts for the high number of falsely positive radionuclide tests (Adolfsson et al., 1982; Edenbrandt et al., 1982 Olsson et al., 1982).

Our finding that the $^{99}\text{Tc}^{\text{m}}$ - plasmin test results successively return to normal in patients with DVT needs to be underlined. This information is of practical importance in evaluating patients who return with symptoms and signs suggestive of acute recurrent DVT. In this situation the $^{99}\text{Tc}^{\text{m}}$ - plasmin test as well as other tests utilizing the same mechanism of action (Ståhlberg, Andersson, Edenbrandt and Strand, 1984) should be evaluated with great care during at least the first six months after a preceding acute DVT.

Acknowledgements

This study has been supported by grants from Thorsten and Elsa Segerfalk's Foundation for Medical Research, Malmöhus Läns Landsting and the Swedish National Association against Heart and Chest Diseases.

References

- Adolfsson L., Nordenfelt I., Olsson H. & Torstensson I. (1982) Diagnosis of deep vein thrombosis with $^{99}\text{Tc}^{\text{m}}$ - plasmin. *Acta Med Scand*, 211, 365-368.
- Armitage P. (1980) Statistical methods in medical research. Blackwell Scientific Publications, Oxford.
- Arnesen H., Hoiseth A. & Ly B. (1982) Streptokinase or heparin in the treatment of deep vein thrombosis. Follow-up results of a prospective study. *Acta Med Scand*, 211, 65-68.
- Becker J., Borgström S. & Saltzman G.-F. (1970) Occurrence and course of thrombosis following prostatectomy. *Acta Radiol Diagnosis*, 10, 513-533.
- Bergvall U. & Hjelmstedt Å. (1968) Recanalization of deep venous thrombosis of the lower leg and thigh. *Acta Chir Scand*, 134, 219-228.
- Common H.H., Seaman A.J., Rosch J., Porter J.M. & Dotter C.T. (1976) Deep vein thrombosis treated with streptokinase or heparin. Follow-up of a randomized study. *Angiology*, 27, 645-654.
- Dahlborn M., Ahlborg G., Söderborg B., Virgin J. & Wilczec B. (1984) Gamma camera detection of $^{99}\text{Tc}^{\text{m}}$ - plasmin in the diagnosis of deep-vein thrombosis. *Eur J Nucl Med*, 9, 499-501.
- Dahn I. & Eiriksson E. (1968) Plethysmographic diagnosis of deep venous thrombosis of the leg. *Acta Chir Scand*, Suppl., 398, 33-42.
- Edenbrandt C.M., Nilsson J. & Ohlin P. (1982) Diagnosis of deep venous thrombosis by phlebography and $^{99}\text{Tc}^{\text{m}}$ - plasmin. *Acta Med Scand*, 211, 59-64.
- Edenbrandt C.M., Dahlström J.A., Nilsson J. & Ohlin P. (1984) Comparison between $^{99}\text{Tc}^{\text{m}}$ - porcine plasmin and $^{99}\text{Tc}^{\text{m}}$ - labelled erythrocytes in diagnosis of deep vein thrombosis. *Clin Physiol*, 4, 243-252.
- Gram J. & Jespersen J. (1984) On the diagnosis of deep venous thrombosis in patients at risk. *Dan Med Bull*, 31, 149-153.
- Gullmo Å. (1956) On the technique of phlebography of the lower limb. *Acta Radiologica*, 46, 603-620.

Hallböök T. & Göthlin J. (1971) Strain gauge plethysmography and phlebography in diagnosis of deep venous thrombosis. *Acta Chir Scand*, 137, 37-52.

Havig Ö. (1977) Deep vein thrombosis and pulmonary embolism. *Acta Chir Scand, Suppl.*, 478.

Hull R. & Hirsh J. (1982) Cost-effectiveness of non-invasive diagnosis of deep vein thrombosis in symptomatic patients. In: *Non-invasive techniques in vascular disease* (ed. E.F. Bernstein), pp. 560-569. C V Mosby Company, London.

Jay R., Hull R., Carter C., Ockelford P., Buller H., Turpie A.G.G. & Hirsh J. (1984) Outcome of abnormal impedance plethysmography results in patients with proximal-vein thrombosis: frequency of return to normal. *Thromb Res.*, 36, 259-263.

Khan O., Ell P.J., Cullum I.D., Jarrit P.H. & Williams E.S. (1981) Clinical and pharmacological studies with $^{99}\text{Tc}^{\text{m}}$ - plasmin. In: *Progress in radiopharmacology* (ed. P.H. Cox), vol 2, pp. 157-171. Elsevier/North Holland Biomedical Press, Amsterdam.

Krohn K.A. & Knight L.C. (1977) Radiopharmaceuticals for thrombus detection: Selection, preparation and critical evaluation. *Sem Nucl Med.*, vol VII, 3, 219-228.

May R. & Nissl R. (1979) *Die Phlebographie der unteren Extremität*. George Thieme Verlag, Stuttgart, FRG.

Nicolaides A.N. & Olsson C.G. (1982) Application of isotope technology to the clinical study of arterial and venous disease. In: *Non-invasive techniques in vascular disease* (ed. E.F. Bernstein), pp. 159-160. C V Mosby Company, London.

Olsson C.G., Albrechtsson U., Darte L. & Persson R.B.R. (1982) $^{99}\text{Tc}^{\text{m}}$ -plasmin for rapid detection of deep vein thrombosis. *Nucl Med Commun*, 3, 41-52.

Rabinov K. & Paulin S. (1983) Venography of the lower extremities. In: *Abrams Angiography; Vascular and Interventional Radiology* (ed. H.L. Abrams), vol 3, pp. 1877-1922. Little, Brown & Company, Boston.

Ståhlberg F., Andersson L., Edenbrandt C.M. & Strand S.E. (1984) Quantitative in vivo and in vitro comparison between radiolabelled colloids, biomolecules and blood cells in their ability to diagnose deep venous thrombosis. *Nucl Med Commun.*, 5, 741-762.

Sumner D.S. (1982) Strain gauge plethysmography. In: *Non-invasive techniques in vascular disease* (ed. E.F. Bernstein), pp. 468-481. C V Mosby Company, London.

Thomas M.L. & McAllister V. (1971) The radiological progression of deep venous thrombus. *Radiology*, 99, 37-40.

Tjen H.S.L.M. (1981) Pathophysiology of thrombus - current clinical requirements for reagents for thrombus detection. In: *Progress in radiopharmacology* (ed. P.H. Cox), vol 2, pp. 103-118. Elsevier/North Holland Biomedical Press, Amsterdam.

Young A.E., Thomas M.L. & Browse N.L. (1974) Comparison between sequelae of surgical and medical treatment of venous thromboembolism. *Brit Med J.*, 4, 127-130.

Zetterqvist S., Ericsson K. & Volpe U. (1975) The clinical significance of biphasic venous emptying curves from the lower limb in venous occlusion plethysmography. *Scand J Clin Lab Invest.*, 35, 497-506.

Table I. Time for normalization of $^{99}\text{Tc}^m$ - plasmin test and maximum venous outflow (MVO) and calf volume expansion (CVE), as measured by strain gauge plethysmography, in three patients with distal DVT (1-3) and five patients with proximal DVT (4-8). Examinations were performed at 1 week, 6 weeks and 6 months after admission. Still pathological tests at the 6 months check-up are indicate (+).

<u>Normalization time (weeks)</u>			
<u>Patients</u>	$^{99}\text{Tc}^m$ - plasmin test	MVO	CVE
1	1	1	1
2	1	1	1
3	26	1	1
4	6	6	6
5	6	6	1
6	26	1	26
7	26	+26	26
8	+26	+26	6

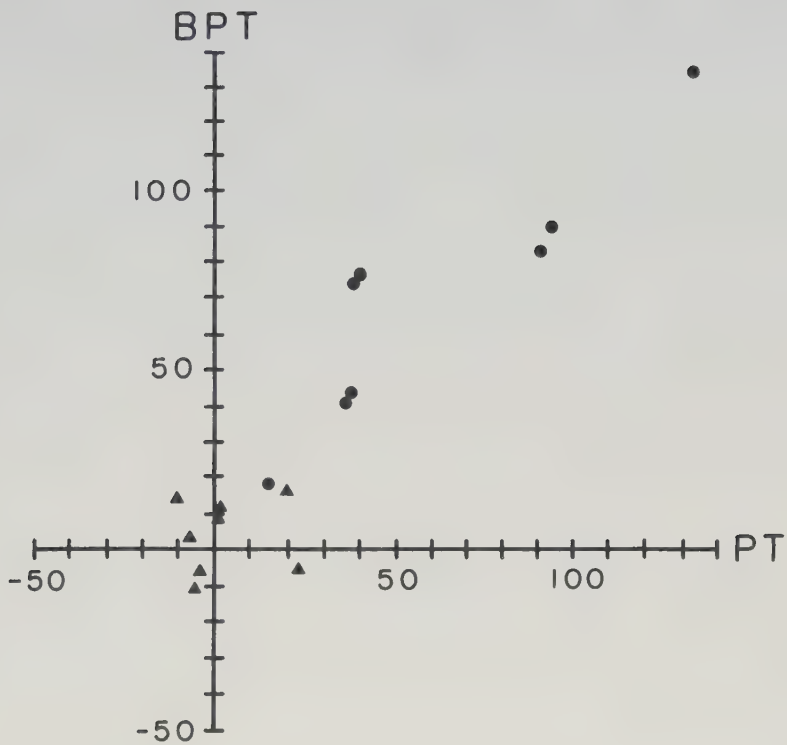


Figure 1. Total predominance of radioactivity in the thrombotic leg (Qsum) calculated from scintillation detector measurements after i.v. injection of $^{99}\text{Tc}^{\text{m}}$ - plasmin (PT) and $^{99}\text{Tc}^{\text{m}}$ - erythrocytes (BPT), respectively, and expressed in 'quotient units' (Olsson et al.,1982). The 'Qsum' of the two tests were compared in eight patients at admission (●) and at six month check-up (▲).

Quantitative *in vivo* and *in vitro* comparison between radiolabelled colloids, biomolecules and blood cells in their ability to diagnose deep venous thrombosis

F. STÅHLBERG¹, L. ANDERSSON¹, C.-M. EDENBRANDT²
and S.-E. STRAND¹

¹Department of Radiation Physics, University of Lund, Sweden

²Department of Medicine, Central Hospital, Helsingborg, Sweden

Received 19 June 1984

Summary

Three radiopharmaceutical groups (colloids, specific biomolecules and blood cells) and a control group were investigated with regard to their ability to rapidly diagnose deep venous thrombosis in an experimental rabbit model. An artificial thrombus was induced in the jugular vein and the radiopharmaceuticals were injected either homolaterally or contralaterally relative to the thrombus. The accumulation of the radioactivity in the thrombus 30 min after the induction was determined *in vivo* from scintillation camera images. After dissection of the jugular vein, the radioactivity of the thrombus was measured *in vitro*.

None of the investigated radiopharmaceutical groups showed any marked high thrombus uptake after contralateral injections, not even the groups that consisted of substances known to be actively involved in coagulation and fibrinolysis. The results exclude a high degree of specific interaction between the radiopharmaceuticals and the thrombus in our model.

After homolateral injection only colloids and reduced ⁹⁹Tc^m-pertechnetate showed a high thrombus uptake, thus also excluding a specific binding to thrombus.

This investigation shows that none of the specific radiopharmaceuticals had a greater ability to accumulate in the thrombus than the colloids, and it is therefore suggested that the clinical usefulness is due to other mechanisms, like circulatory changes secondary to the DVT.

Introduction

Diagnosis of deep venous thrombosis (DVT) in the lower extremities is a frequent problem in patients admitted to the medical wards and essential due to the risk of

fatal pulmonary embolism [1]. Confidence in purely clinical observations for diagnosis is still common [2] although this method has been shown to be unreliable [3]. Phlebography is hitherto the most widely used diagnostic method but it is inconvenient and painful to the patient and it may cause thrombosis [4,5]. Furthermore it is expensive [6] and involves an effective dose equivalent of about 1.5 mSv [7]. During the last two decades a considerable number of radiopharmaceuticals have been used as less traumatic and less expensive alternatives. However, since the mechanisms for accumulation of radiopharmaceuticals in the diseased leg of patients with suspected DVT are not known and clinical studies reported in the literature differ in arrangements and results, there has been no consensus in the selection of a suitable radiopharmaceutical for a convenient screening method for DVT.

The aim of the present study was to compare the uptake at the thrombus site of three groups of radiopharmaceuticals outgoing from an experimental model in the rabbit. The biomolecule group consisted of substances supposed to be actively involved in coagulation and fibrinolysis. This group was studied in order to elucidate whether a rapid specific interaction with the thrombus is of significance [8–10]. It has also been put forward that merely mechanical entrapment is responsible for the localization of the radiopharmaceutical [11], and, in order to investigate this hypothesis, colloids of different particle sizes were tried. Furthermore, labelled blood cells, which have recently been reported to be useful in the diagnosis of DVT [12–16], were evaluated since they are supposed both to reflect the haemodynamic changes at the thrombus site and to be trapped in the thrombus.

In the clinical situation, the radiopharmaceutical is either injected into a dorsal foot vein distal to the thrombus, γ -phlebography, or into an antecubital vein for further transport by the general blood circulation to the thrombus site. Disparities of clinical reports regarding the same radiopharmaceutical could be dependent upon the injection site and therefore homolateral as well as contralateral injection relative to the thrombus was tested in the animal model.

Due to the fact that each animal experiment takes about three days to fulfil, the aim has not been to collect enough data for a statistically correct evaluation substance by substance, but rather to make possible a meaningful comparison group by group.

Methods

RADIOPHARMACEUTICALS

Fourteen radiopharmaceuticals were investigated and divided into four main groups: colloids, biomolecules, blood cells and control substances. The investigated radiopharmaceuticals are listed in Table 1 together with their main characteristics.

Colloids

Antimony colloid, tin-sulphur colloid and human serum albumin colloid were received as

Table 1. The fourteen radiopharmaceuticals studied. Thirty-six experiments were performed (*N*). Labelling efficiency was determined by gel chromatography column scanning of control substances, colloids and biomolecules, and by separation of cells and plasma fraction for blood cells. Particle size of colloids was determined by ultrafiltration. Data obtained from literature are indicated.

Radio-pharmaceuticals	N	Mean labelling efficiency (%)		Mean particle size (nm)	Reported studies in literature	
		Sephadex G-25	Sepharose 4B		Animal/patient*	Reference
<i>Control substances</i>						
⁹⁹ Tc ^m O ₄ ⁻ (pertechnetate)	2	95	95	0, 5 [23, 24]	P	[37]
⁹⁹ Tc ^m O ₄ ⁻ + Sn ²⁺ (reduced pertechnetate)	2	≤ 95†	85	—	—	
⁹⁹ Tc ^m -Human serum albumin	2	85	85	MW 65 000 [41]	—	
<i>Colloids</i>						
⁹⁹ Tc ^m Sb ₂ S ₃ (antimony colloid)	2	≤ 95†	95	40	—	
⁹⁹ Tc ^m (Sn)S (tin-sulphur colloid)	2	≤ 95†	55	40	—	
⁹⁹ Tc ^m ₂ S ₇ (sulphur colloid)	2	≤ 95†	≤ 50†	300	A, P	[29–31]
⁹⁹ Tc ^m -Human serum albumin microcolloid	2	≤ 80†	≤ 70†	500	P	[7]
⁹⁹ Tc ^m -Macro-aggregated human serum albumin	2	—	—	20 000‡	A, P	[28, 42]
<i>Biomolecules</i>						
⁹⁹ Tc ^m -Porcine plasmin	7	60	65	MW 80–100 000 [43]	A, P	[18, 33, 34]
⁹⁹ Tc ^m -Human fibrinogen	3	75	55	MW 400 000 [41]	A	[9]
¹³¹ I-Dansyl-cadaverine	2	—	—	—	—	[44]
<i>Blood cells</i>						
		Centrifugation				
⁹⁹ Tc ^m -Erythrocytes	2	95	—	—	P	[12, 13]
¹¹¹ In-Platelets	4	80	—	—	A	[14, 15, 45]
¹¹¹ In-Leukocytes	2	60	—	—	A	[16]

*A, animal; P, patient.

†For this particle size the gel resolution limit is exceeded and large-sized impurities might be included in the peak area.

‡L. Duarte (personal communication).

prefabricated kits. Sulphur colloid was manufactured according to Persson and Naversten [17]. Also included in this group was prefabricated macroaggregated human serum albumin.

Biomolecules

Porcine plasmin, human fibrinogen and dansylcadaverine were received as prefabricated kits.

Blood cells

Erythrocytes, leukocytes and platelets were isolated from whole blood withdrawn from the rabbits immediately before the experiments.

Control substances

$^{99}\text{Tc}^{\text{m}}$ -Pertechnetate ($^{99}\text{Tc}^{\text{m}}\text{O}_4^-$) obtained from a $^{99}\text{Mo}/^{99}\text{Tc}^{\text{m}}$ -generator (Byk-Mallinckrodt), reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate ($^{99}\text{Tc}^{\text{m}}\text{O}_4^- + \text{Sn}^{2+}$) produced as described by Persson *et al.* [18], and human serum albumin from a prefabricated kit were investigated as control substances.

Reference substance

The radiopharmaceuticals were in all experiments compared with a reference substance, ^{125}I -fibrinogen (Radiochemical Centre, Amersham, UK).

LABELLING METHODS

Colloids, erythrocytes and biomolecules except dansylcadaverine were labelled with pertechnetate. In all the kits stannous ion was used as reducing agent. Dansylcadaverine was labelled by the manufacturer with ^{131}I . Leukocytes and platelets were labelled with ^{111}In -oxinate according to Olsson [19].

QUALITY CONTROL

The labelling efficiency of the colloids and biomolecules were controlled by means of gel chromatography column scanning (GCS) [20–22]. Both Sephadex G-25 fine and Sepharose 4B gels (Pharmacia Fine Chemicals, Uppsala, Sweden) were used. The labelling efficiency of the blood cells, obtained by centrifugation expresses the activity in the blood cells out of the total activity in the blood.

The particle size distribution was studied using three different techniques. The GCS method was used for a qualitative particle size estimation in the size range of 0.1 nm to 0.1 μm . Photon correlation spectroscopy (Nanosizer, Coulter, UK) and ultrafiltration with polycarbonate filters (Nuclepore Corp., USA) were used for quantitative particle size distribution measurements of colloids in the size range of 20 nm to 3 μm and 30 nm to 1 μm , respectively [20]. The particle size of $^{99}\text{Tc}^{\text{m}}$ -macroaggregated albumin have previously been determined to be 10–80 μm with a mean of 20 μm (L. Duarte, personal communication). The effective ionic diameter of $^{99}\text{Tc}^{\text{m}}$ -pertechnetate was calculated to be about 0.5 nm from data published by Cobble [23] and Boyd [24].

ANIMAL MODEL

The experiment was performed in a total of 36 rabbits, with a mean weight of 2.7 ± 1.4 (S.D.) kg. The rabbits were anaesthetized with pentobarbital (Mebumal, ACO, Sweden) and placed in a supine position. Carbonyl iron (100 mg) was injected into an ear vein. Injections of KI (10 mg), for thyroid blocking, and ^{125}I -fibrinogen (1 MBq), as a reference substance, were made 15 min prior to the injection of the iron suspension. The iron particles were trapped in the external

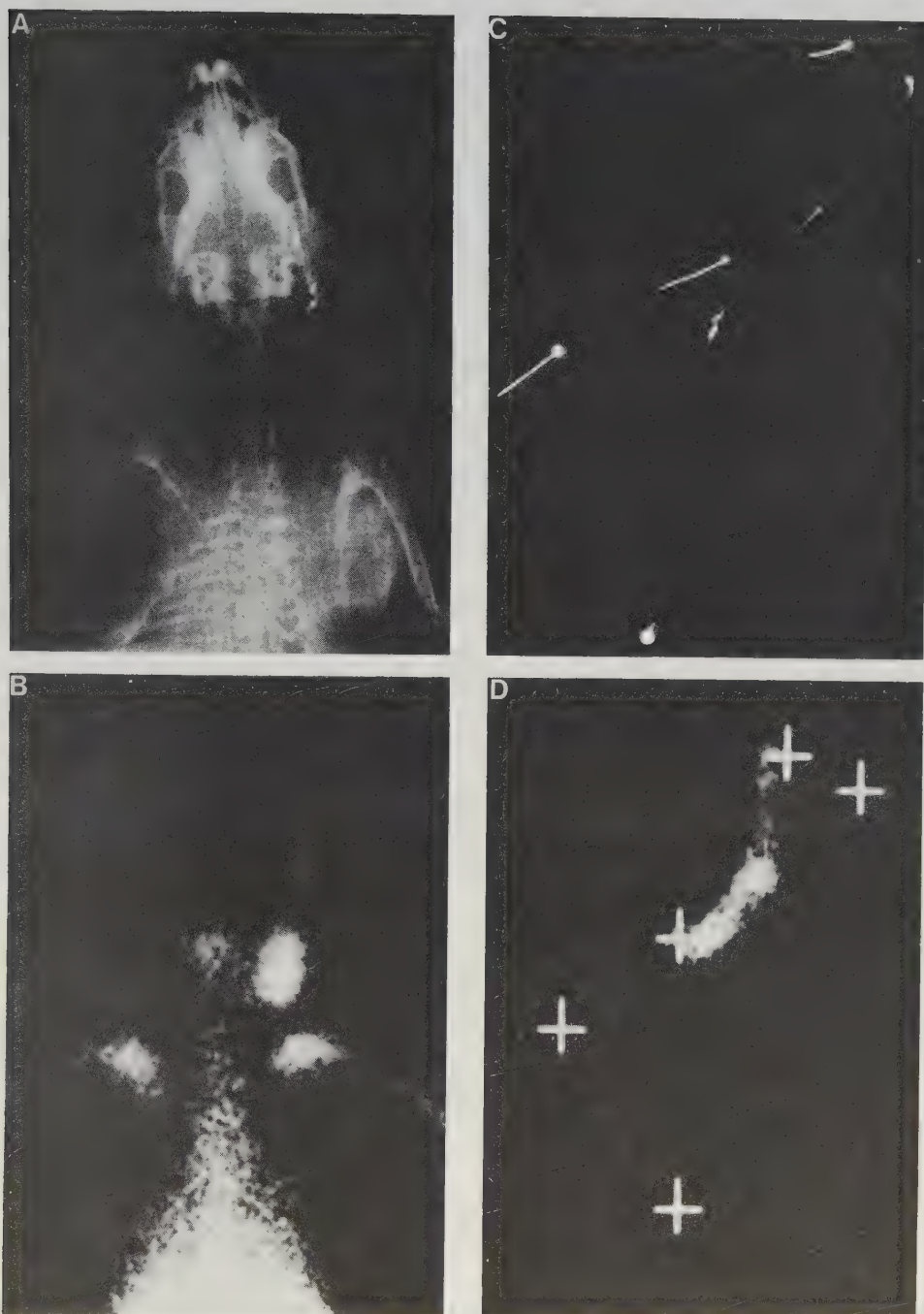


Fig. 1. The experimental model for induction and detection of DVT in rabbits. (Investigated substance, $^{99}\text{Tc}^{\text{m}}$ plasmin). (A) X-ray picture showing the iron suspension trapped in the right jugular vein of a rabbit. (B) Scintillation camera image showing the *in vivo* uptake of radioactivity. (C) X-ray picture obtained after dissecting out the jugular vein showing the trapped iron particles and the pins used to fasten the vessel. (D) Scintillation camera image of the same vessel specimen as shown in (C). The positions of the pins are electronically marked.

jugular vein by the magnetic field (0.1 T) of a small magnet. After 30 min, the magnet was removed and the fixation of the trapped iron particles in the jugular vein was verified by 60 kV X-ray imaging (Fig. 1A). When the iron entrapment was confirmed and a scintillation camera image of the ^{125}I -fibrinogen distribution was taken, about 30 min after the removal of the magnet, 1.0 ml (about 40 MBq for $^{99}\text{Tc}^m$ -substances and 5–10 MBq for ^{125}I , ^{131}I , and ^{111}In substances) of the radiopharmaceutical under investigation was injected into an ear vein. Each radiopharmaceutical, except $^{99}\text{Tc}^m$ -macroaggregated albumin, was in one rabbit injected in the same ear as the iron particles (homolaterally) and in a second rabbit in the ear opposite to the iron particles (contralaterally). $^{99}\text{Tc}^m$ -Macroaggregated albumin was only injected homolaterally. $^{99}\text{Tc}^m$ -Fibrinogen was in an additional control experiment injected together with ^{125}I -fibrinogen prior to the formation of the thrombus.

After the *in vivo* measurements 3.0 ml blood was withdrawn from the heart of the rabbit and immediately thereafter the rabbit was killed. The vein containing the thrombus was subsequently dissected out.

ACTIVITY UPTAKE MEASUREMENTS

The uptake of the radiopharmaceutical at the thrombus site was determined *in vivo* about 30 min after the injection using a scintillation camera (Pho Gamma IIIHP, Searle). The image was taken as an anterior view of the rabbit with five min exposure time (Fig. 1B). Identical regions of interest were chosen on the thrombus side and on the opposite side of the rabbit's neck and the respective count rates per picture element in the chosen region of interest were tabulated. The count rates determined at the thrombus region and at the opposite side of the rabbit's neck, regarding the radiopharmaceutical investigated, were used to calculate a thrombus-to-background uptake ratio (T/B) for each radiopharmaceutical. The scintillation camera images were recorded on Polaroid films. The visibility of the radioactivity in the thrombus area was evaluated using a three-graded scale.

After having killed the rabbit the jugular vein containing the palpable thrombus was dissected out for further measurements *in vitro*. The iron entrapment was localized by X-ray imaging of the vessel (Fig. 1C). The vessel was thereafter placed on the scintillation camera for a 5 min static image (Fig. 1D). By means of these images the blood vessel was cut into pieces taking into consideration the localization of iron particles, the radioactivity distribution and the vessel bifurcations. The vessel specimen and the blood sample withdrawn from the rabbit were analysed using a well counter (Selectronic, Nuclear Data). The specific count rate determined for each vessel piece and for the blood sample regarding both the radiopharmaceutical investigated and ^{125}I -fibrinogen were used to calculate an *in vitro* thrombus-to-blood uptake ratio ($\hat{T}/\hat{B}$) for each piece of vessel. The thrombus-to-blood uptake ratios of the radiopharmaceutical were hereafter normalized to the $\hat{T}/\hat{B}$ ratios of the ^{125}I -fibrinogen, obtaining the ratios R in order to correct for differences of thrombi between the experiments:

$$R(\text{investigated substance}) = \frac{\hat{T}/\hat{B} (\text{investigated substance})}{\hat{T}/\hat{B} (^{125}\text{I-fibrinogen})}$$

Finally, the *in vitro* count rates were used in order to calculate specific activity in thrombus and blood samples in percentage of injected activity.

The total time needed for one experiment was approximately three days.

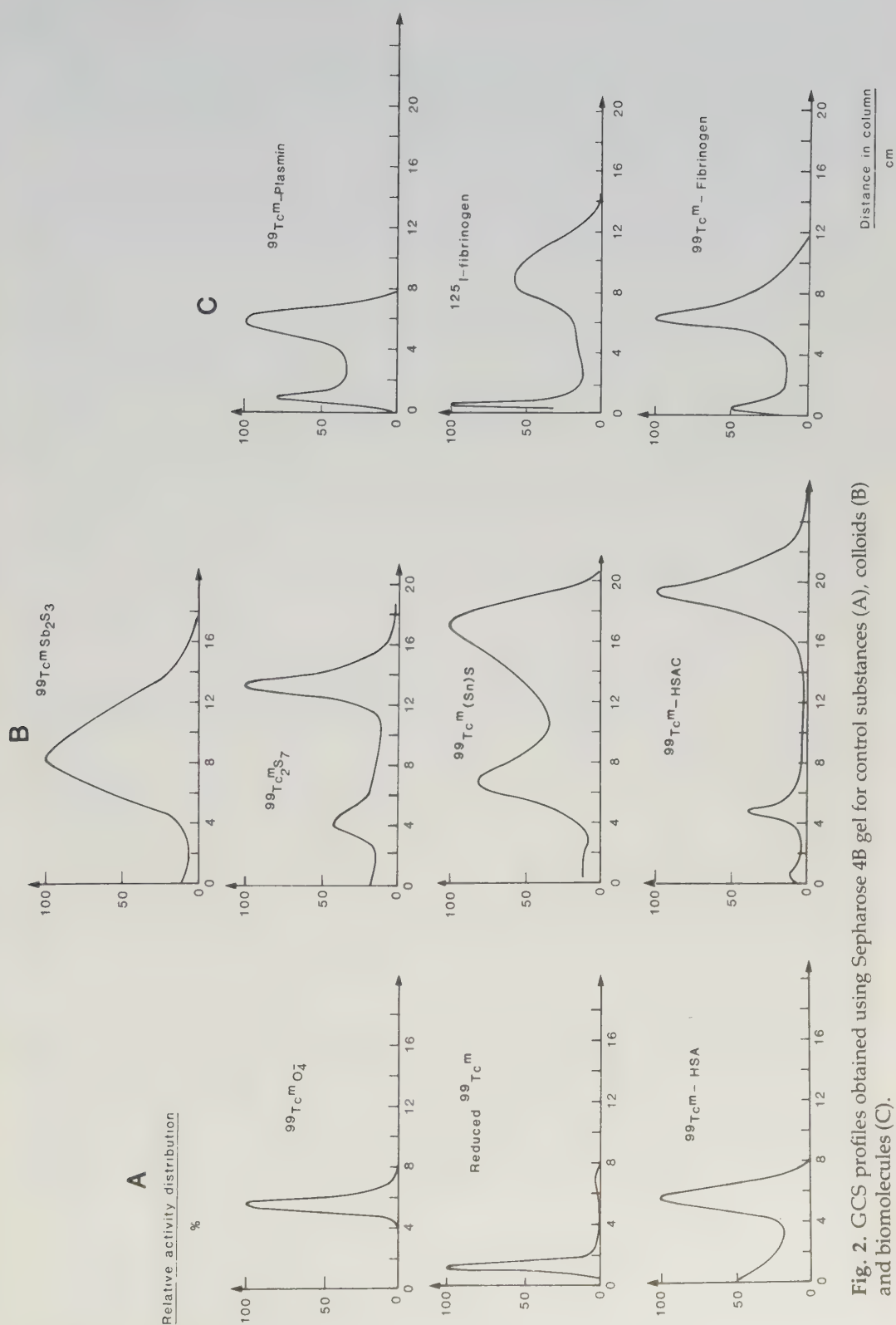


Fig. 2. GCS profiles obtained using Sepharose 4B gel for control substances (A), colloids (B) and biomolecules (C).

STATISTICAL METHODS

Calculations of the estimated total error in the T/B , $\hat{T}/\hat{B}$, R and percentage specific activity measurements were made for each experiment. The total error was considered to consist of a Poisson counting statistical error (± 2 S.D.), an error due to difficulties in identifying the region of interest over the thrombus region (T/B) and an error in the measurement of total injected activity (percentage specific activity measurement).

The error introduced in the $\hat{T}/\hat{B}$ ratio and in the measurements of percentage specific activity in the thrombus by the presence of iron particles in some of the vessel specimens, influencing specimen weight (about 50–200 mg) was uncorrected and was not estimated. However, these results were supplemented by the corresponding R ratios which are independent of sample weight.

As mentioned earlier a statistical analysis substance for substance is not possible to perform due to the low number of experiments per substance. However, in the case of $^{99}\text{Tc}^{\text{m}}$ -plasmin five homolateral and three contralateral experiments were performed, and the corresponding errors (± 2 S.D.) have been added in order to show the approximate size of the biological variations involved in the experiments. Furthermore, the results can be compared group by group and for this analysis two nonparametric tests, the Mann–Whitney U -test for comparison between different groups and the sign test for comparison within a group, were used. The significance levels were chosen as $P > 0.95$ and $P > 0.90$ [25]. (Within these limits, the colloid group was the only group large enough to give significant test results when the sign test was used.)

Results

Quality control

The labelling efficiencies of the radiopharmaceuticals appear from Table 1. Lower labelling efficiencies were often found when using Sepharose 4B compared with Sephadex G-25 fine gel for testing colloids and biomolecules. Individual profiles appear in Fig. 2. It should be noted that the GCS profiles also reflect the particle size of the radiopharmaceutical [21].

The cumulative size distribution of colloids in the size range 30 nm to $1\mu\text{m}$, determined by ultrafiltration, is shown in Fig. 3. The mean particle sizes of the colloids were estimated from this figure to be between 40–500 nm (Table 1). The mean diameters of the colloids as determined by photon correlation spectroscopy showed similar values as determined by the ultrafiltration technique and are indicated in Fig. 3 with arrows.

Activity uptake measurements

The thrombus-to-background uptake ratio (T/B), according to the *in vivo* measurements and the graded visibility of the radioactivity at the thrombus site on Polaroid films, are shown for each radiopharmaceutical in Fig. 4. In this and the following figures the mean values for each substance is presented if more than one experiment has been performed. As can be seen the values ranged from 0.4 to 16 and a low T/B ratio ($T/B < 2.2$) was found for all radiopharmaceuticals after contralateral injection,

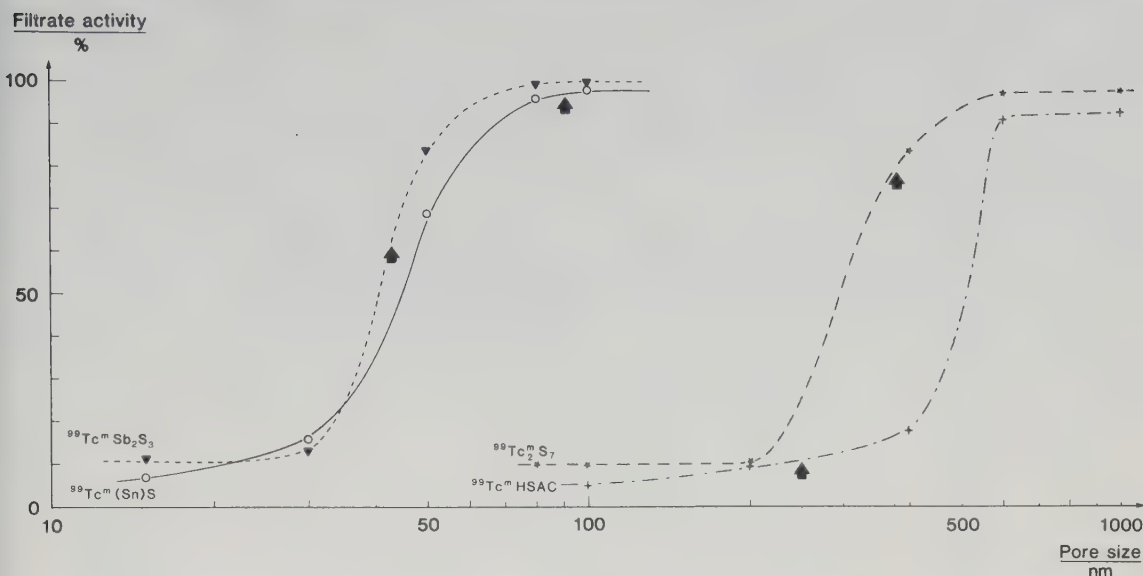


Fig. 3. Particle size distribution showed by percentage activity passing polycarbonate filters with different pore sizes. $^{99}\text{Tc}^m$ -Antimony colloid ($\text{---}\blacktriangledown\text{---}\blacktriangledown\text{---}$), $^{99}\text{Tc}^m$ -tin-sulphur colloid ($\text{---}\text{O}\text{---}\text{O}\text{---}$), $^{99}\text{Tc}^m$ -sulphur colloid ($\text{---}\text{---}\text{---}$) and $^{99}\text{Tc}^m$ -human serum albumin microcolloid ($\text{---}\cdot\text{---}\cdot\text{---}$) were investigated. Arrows indicate mean particle size as measured by photon correlation spectroscopy.

while the radiocolloids show markedly higher T/B ratios ($T/B = 5.2\text{--}9.3$) than the other groups after homolateral injection except reduced $^{99}\text{Tc}^m$. The visibility of the radioactivity at the thrombus site was well in accordance with the T/B ratio calculated from the scintillation camera images.

The thrombus-to-blood uptake ratios ($\hat{T}/\hat{B}$) according to the *in vitro* measurements for the radiopharmaceutical investigated are given in Fig. 5 together with the R ratios for each piece of vessel. A high $\hat{T}/\hat{B}$ ratio was found for vessel specimens distal to the iron entrapment regarding radiocolloids, ^{111}In -leukocytes and reduced $^{99}\text{Tc}^m$ -pertechnetate injected homolaterally. The other seven radiopharmaceuticals showed a low $\hat{T}/\hat{B}$ ratio after homolateral injection. After contralateral injection all radiopharmaceuticals showed low $\hat{T}/\hat{B}$ ratios.

The vessel piece with the highest R ratio in each experiment, provided that it did not lie more than one bifurcation away from the position of the iron, was picked out for comparison between radiopharmaceuticals and are shown in Fig. 6. The R values spread from 0.3 to 375. Side vessels to the external jugular vein were also studied but showed in no case higher R ratios. After homolateral injection high R ratios ($R = 12\text{--}375$) were found only for the radiocolloids. After contralateral injection all R ratios

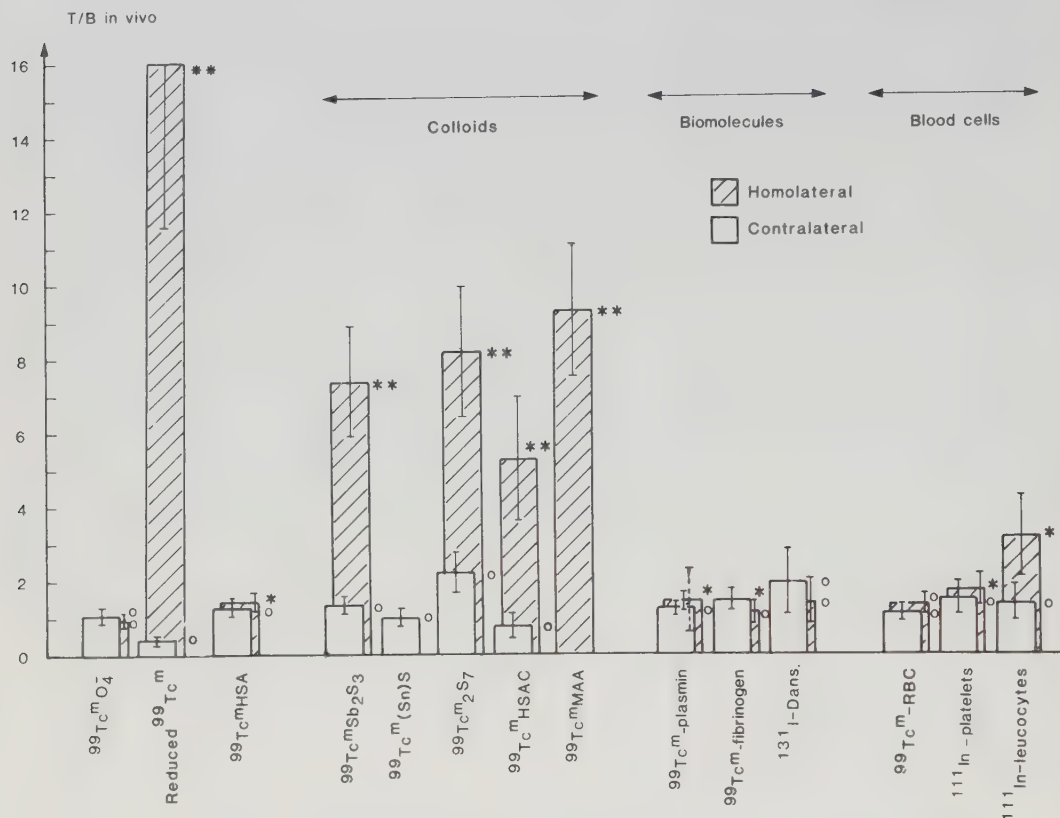
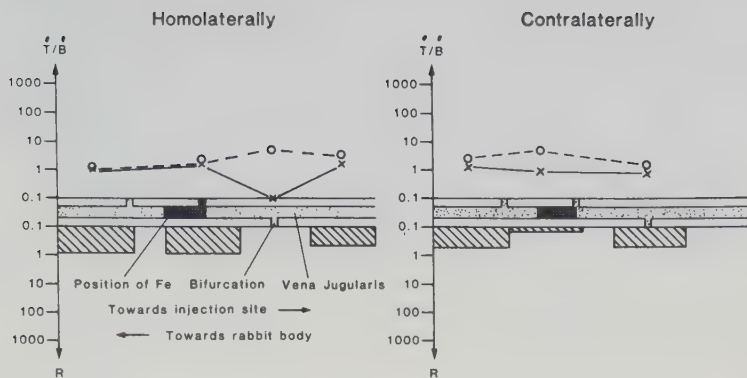
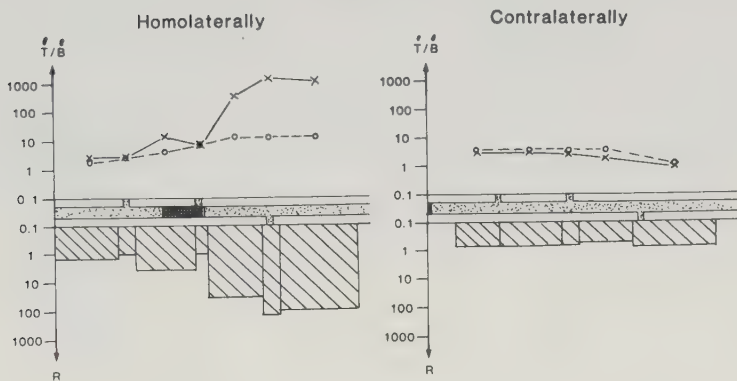
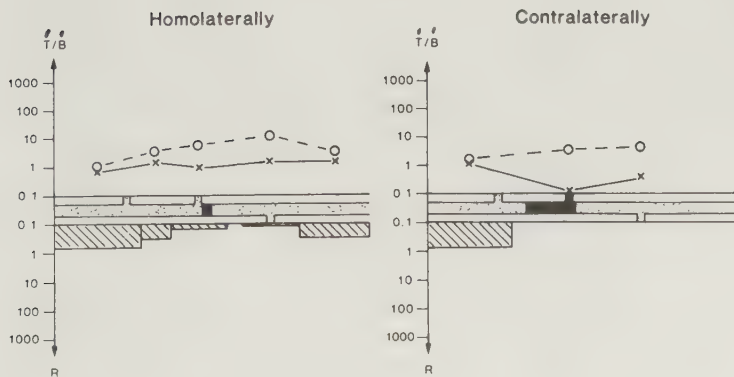
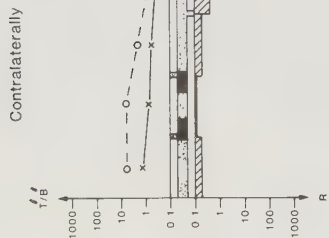
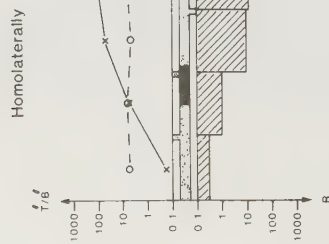
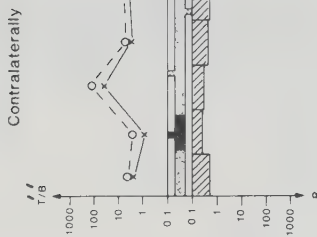
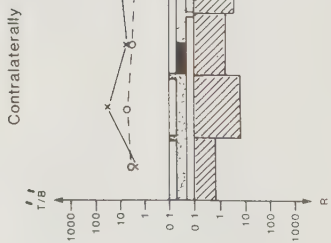
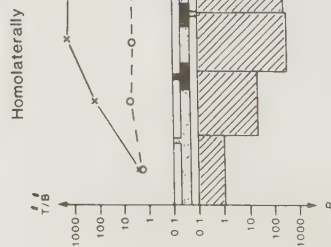
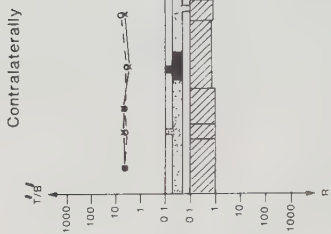
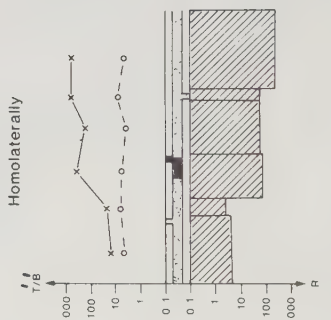
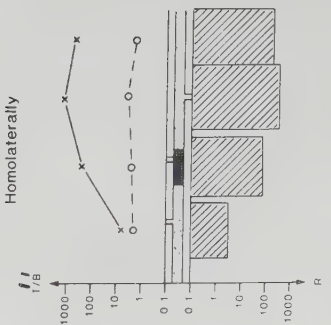


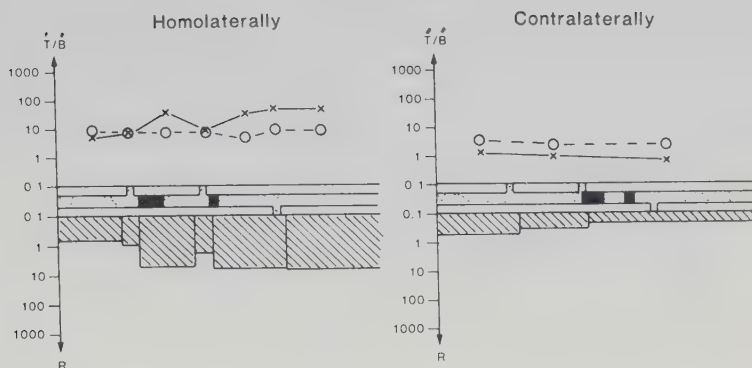
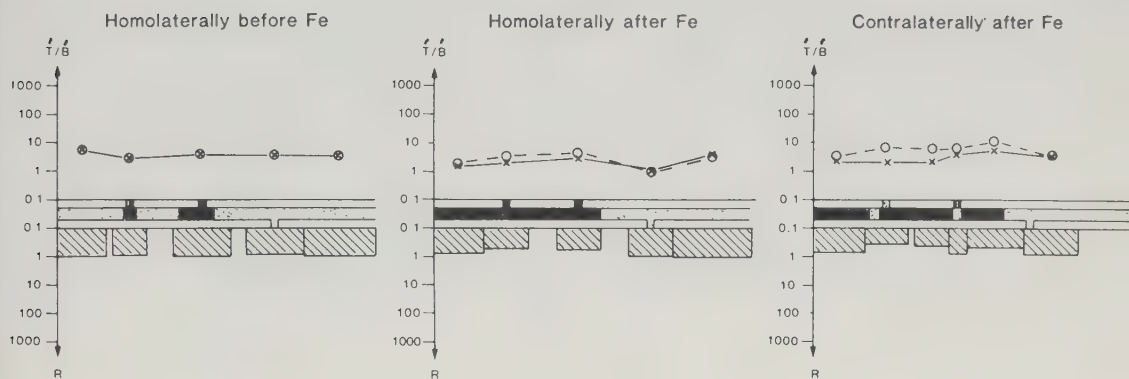
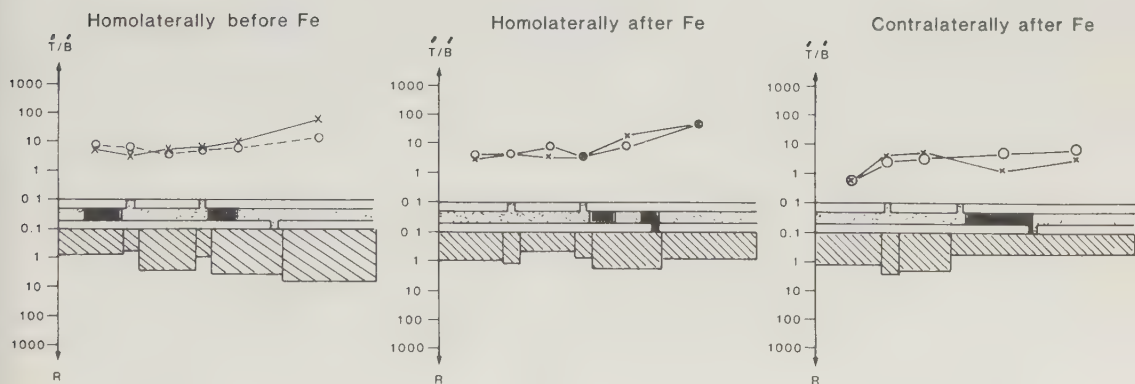
Fig. 4. The thrombus-to-background ratio (T/B) determined after *in vivo* measurements. The error margins indicate the total estimated error. For $^{99}\text{Tc}^{\text{m}}\text{-plasmin} \pm 2 \text{ S.D.}$ based on repetitive experiments are added, $n = 5$ homolaterally and $n = 3$ contralaterally. The visibility of the radioactivity at the thrombus site on analogue images is indicated according to the following scale: **, good visualization; *, fair visualization; o, no visualization. ▨, injection homolaterally to the thrombus; □, injection contralaterally to the thrombus.

Fig. 5. The thrombus-to-blood uptake ratio $\hat{T}/\hat{B}$ determined *in vitro* for the radiopharmaceuticals investigated (x) and for the reference substance $^{125}\text{I}\text{-fibrinogen}$ (o) together with the corresponding R ratios (■), in each piece of the jugular vein. The position of the iron particles (■) and the vessel bifurcations are indicated. The injection site was in an ear vein to the right of (distally to) the iron particles. (A) Control substances, (B) colloids, (C) biomolecules and (D) blood cells.

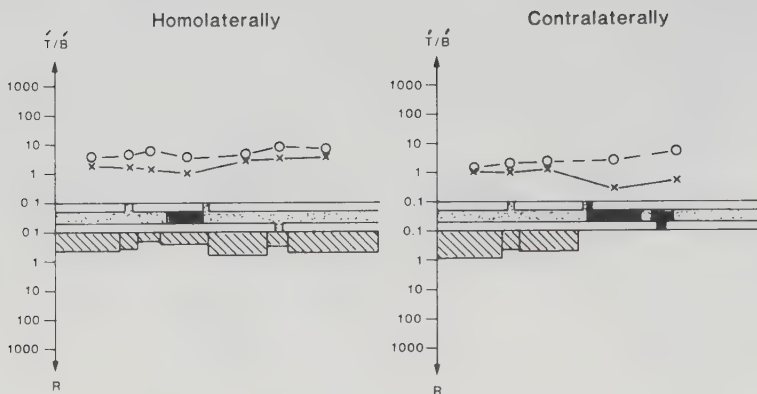
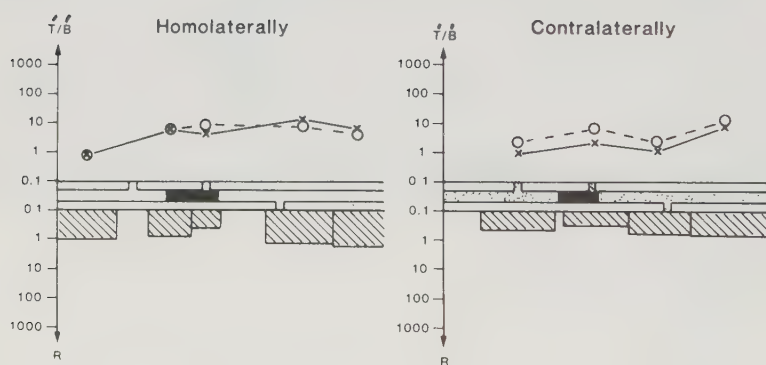
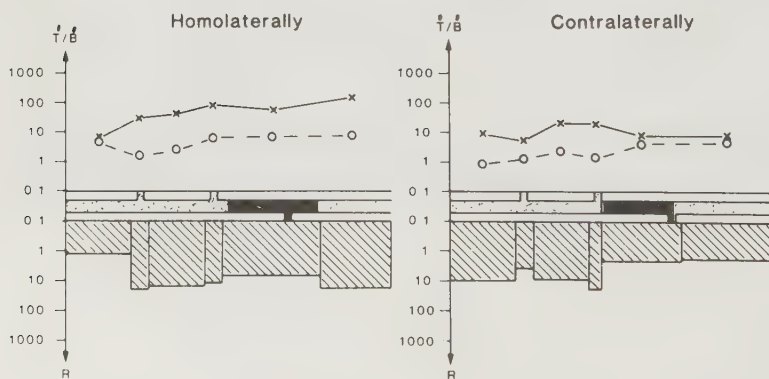
5A

 $^{99}\text{Tc}^{\text{m}}\text{O}_4^-$ Reduced $^{99}\text{Tc}^{\text{m}}$  $^{99}\text{Tc}^{\text{m}}\text{-HSA}$ 

$^{99}\text{Tc}^m\text{Sb}_2\text{S}_3$  $^{99}\text{Tc}^m(\text{Sn})\text{S}$  $^{99}\text{Tc}^m\text{S}_2\text{S}_7$  $^{99}\text{Tc}^m\text{-HSAC}$  $^{99}\text{Tc}^m\text{-MAA}$ 

 $^{99}\text{Tc}^{\text{m}}$ - Fibrinogen ^{131}I -Dansylcadaverine

5D

 $^{99}\text{Tc}^m - \text{RBC}$  $^{111}\text{In} - \text{Platelets}$  $^{111}\text{In} - \text{Leucocytes}$ 

were low ($R = 0.2-6$) and the *in vivo* and *in vitro* results are thus in good agreement.

Based upon the *in vitro* measurements of the vessel pieces with the highest R ratios and of the blood samples the specific activity in thrombus and in blood was calculated and is shown for each substance in Fig. 7A,B as a percentage of injected activity. The percentage specific activities range in the thrombus between 0.005 and 66% g^{-1} and in the blood between 0.006 and 0.6% g^{-1} . A high specific activity in the

Table 2a. Results of Mann-Whitney U -test on the parameters T/B , R and percentage specific activity in two-by-two comparisons between the three groups radiocolloids (RC), biomolecules (BM) and blood cells (BC).

	RC-BM	RC-BC	BM-BC
T/B			
Homolaterally	C > BM*	RC > BC*	n.s.
Contralaterally	n.s.	n.s.	n.s.
R			
Homolaterally	RC > BM†	RC > BC*	n.s.
Contralaterally	n.s.	n.s.	n.s.
% Specific activity			
Thrombus homolaterally	RC > BM†	n.s.	BM < BC*
Thrombus contralaterally	n.s.	RC < BC*	n.s.
Blood	n.s.	RC < BC†	n.s.

* $P > 0.09$.

† $P > 0.95$.

thrombus was noted for colloids (1.3–57% g^{-1}) after homolateral injection and for reduced $^{99}\text{Tc}^{\text{m}}$ (66% g^{-1}). The lowest specific uptake in blood was also noted for the colloids (0.006–0.04% g^{-1}).

In Table 2a and b, a summary of the statistical evaluations of the results from Figs 4, 6 and 7 are given.

From Table 2a it is seen that the colloids are significantly separated from the biomolecules and the blood cells with regard to T/B and R values. For percentage specific activity in thrombus homolaterally radiocolloids and blood cells significantly differs from biomolecules, and for percentage specific activity in blood radiocolloids differs significantly from blood cells.

From Table 2b it is seen that, for the colloid group, there is a significant difference between homolateral and contralateral injection site with regard to R and a percentage specific activity in thrombus, and that the percentage specific activity uptake in the thrombus significantly differs from the uptake in the blood after homolateral injection.

Table 2b. Results of the sign test on the parameters T/B , R and percentage specific activity in a comparison between homolateral (H) and contralateral (C) injection and on percentage specific activity in a comparison between uptake in thrombus (T) and uptake in blood (B) for the colloid group. — indicates too few observations to reach significance.

	$H-C$	$T-B$
T/B	—	
R	$H > C^*$	
% specific activity		
Thrombus	$H > C^*$	} $H:T > B^*$ C: n.s.
Blood	n.s.	

* $P > 0.90$.

Discussion

Fundamental to a proper evaluation of radiopharmaceuticals aimed to diagnose DVT is the radiochemical purity [26]. Gel chromatography column scanning has been shown to be a valuable method to determine such radiochemical impurities [21]. The necessity to select an appropriate gel for quality control tests has recently been emphasized [22] and confirmed by our studies. Our results showed a high labelling yield although less than 100%. This might introduce some uncertainty as to what component in the injected preparation that caused the activity uptake in the thrombus. Using both $^{99}\text{Tc}^{\text{m}}$ -pertechnetate and reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate as control substances in the experiments, however, enhanced the possibility of evaluating the uptake of radiochemical impurities in the thrombus. Nevertheless, all the quality control tests were performed *in vitro* and consequently some uncertainty about the radiochemical purity *in vivo* still remains [26].

In order to show the thrombus buildup and to standardize for differences of thrombi between the experiments, ^{125}I -fibrinogen was injected as a reference substance prior to the thrombus induction. The specific accumulation of ^{125}I -fibrinogen in the thrombus was shown by the thrombus-to-blood ratio ($\hat{T}/\hat{B}$) [27] and was similar to the accumulation of $^{99}\text{Tc}^{\text{m}}$ -fibrinogen.

In the present study the radiopharmaceuticals were injected after thrombus induction and the results can consequently be compared with clinical experiences of diagnosing 'established' DVT. The selection of 30 min as observation time for all

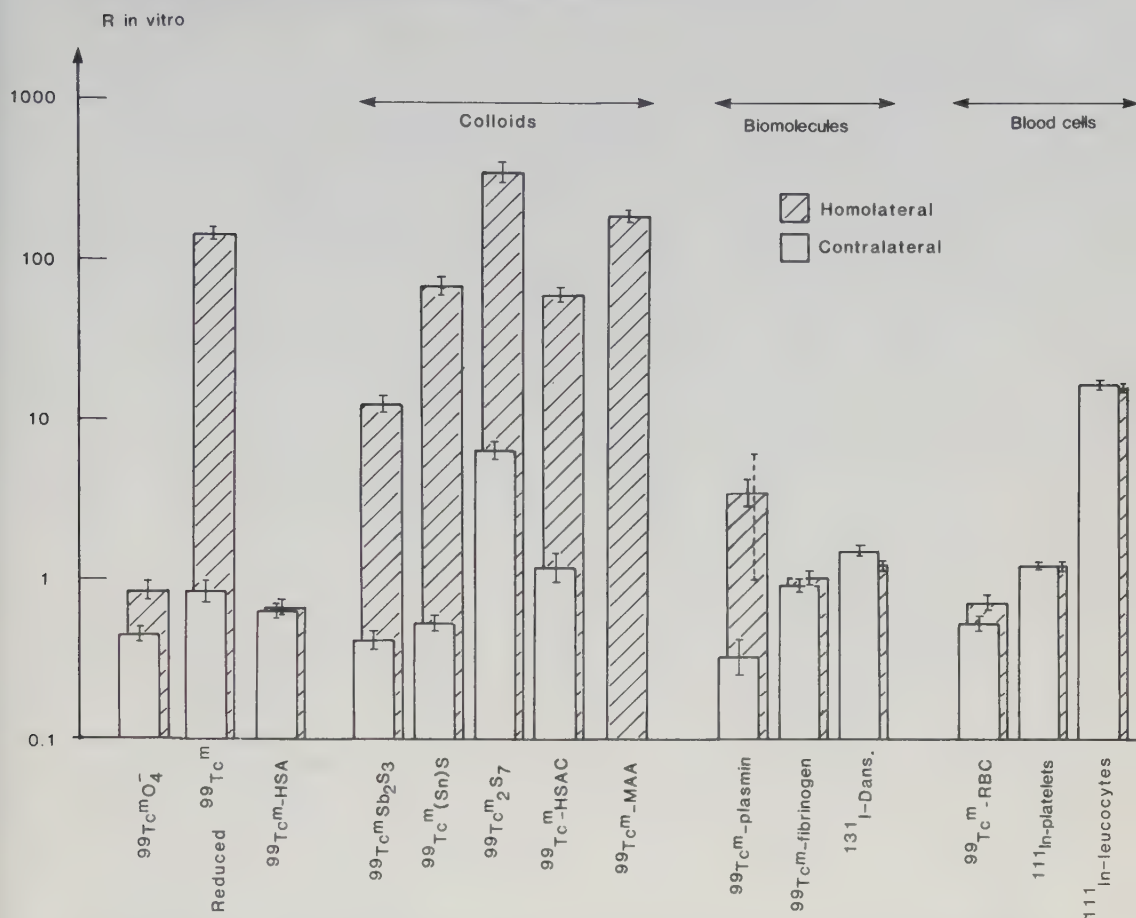


Fig. 6. The ratio (R) between the thrombus-to-blood uptake ratio ($\hat{T}/\hat{B}$) for each the radiopharmaceutical and the corresponding ($\hat{T}/\hat{B}$) ratio for ^{125}I -fibrinogen as calculated after both homolateral (▨) and contralateral (□) injection. The estimated errors indicated are the same as in Fig. 4.

radiopharmaceuticals was motivated by our ambition to study uptake processes useful for rapid detection of DVT.

Radiolabelled colloids showed, in comparison with the other groups, a significant accumulation at the thrombus site after homolateral injection, both *in vivo* and *in vitro*. This is well in accordance with 'hot-spots' in association with DVT found after injection of radiopharmaceuticals distal to the thrombus γ -phlebography [28]. A low difference in radiocolloid uptake between the thrombotic leg and the healthy leg has also been found in clinical studies after injection of the radiopharmaceutical in an antecubital vein [29–31]. The present results favour an injection site distal to the

A

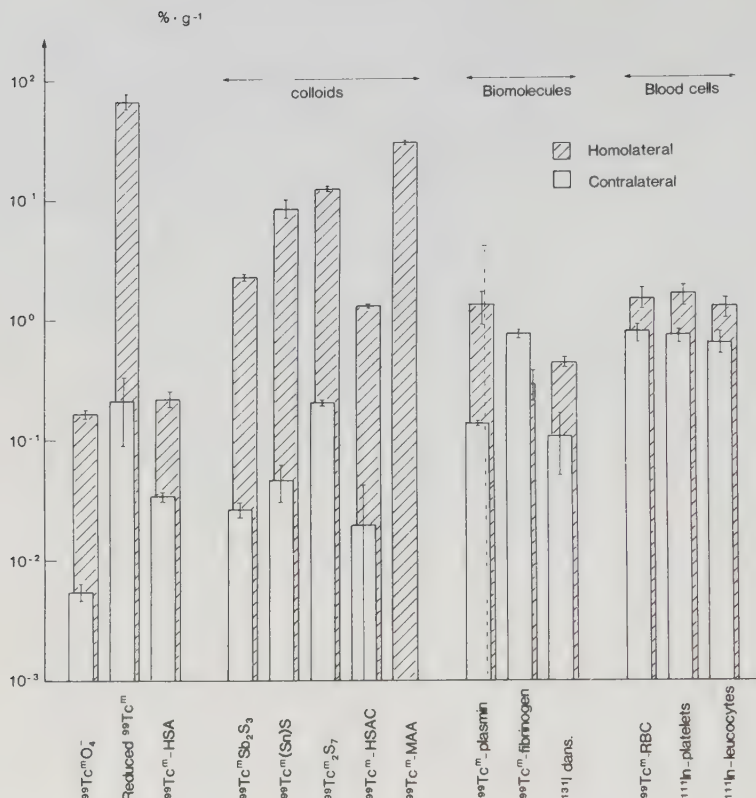
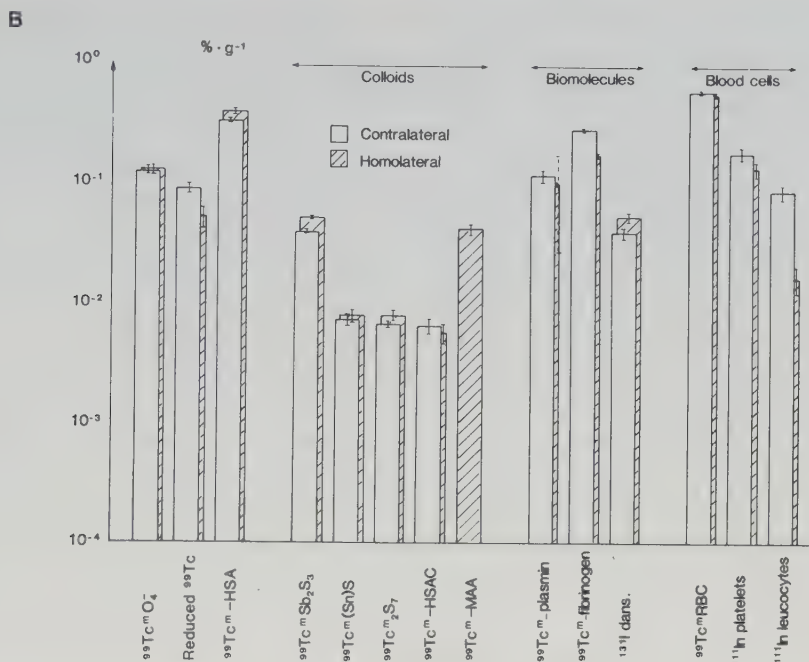


Fig. 7A and B. Specific activity in the thrombus (A) and in blood (B) as percentages of injected activity after homolateral (▨) and contralateral (□) injection. The estimated errors indicated are the same as in Fig. 4.

thrombus but contrary to our expectations, particle size was not a factor of significance for the thrombus uptake. The reason for any accumulation of radiolabelled colloids at the thrombus site is still unknown, but different hypotheses as electrostatic forces, adhesion, mechanical entrapment [11] and reflection of venous stasis [32] have been proposed.

Radiolabelled biomolecules showed only a slight accumulation at the thrombus site after both homolateral and contralateral injections, measured *in vivo* as well as *in vitro*. The low thrombus uptake of particularly ⁹⁹Tc^m-porcine plasmin contralaterally questions any significant specific interaction between this radiopharmaceutical and the thrombus to cause an increased accumulation of radioactivity in the thrombotic leg [33–35]. The ⁹⁹Tc^m-plasmin test seems rather to utilize mechanisms



not specifically evaluated in the present study, such as circulatory changes secondary to the DVT [36].

Regarding the blood cell group, ¹¹¹In-leucocytes showed an increased *T/B* ratio mainly after homolateral injection, and a further study in order to establish this result statistically would be of interest.

In the control group a low activity uptake was shown after contralateral injection. A clinical study performed on ⁹⁹Tc^mO₄⁻ has also not been able to show a particular thrombus uptake but rather a nonspecific accumulation of low magnitude in the diseased leg [37].

In the homolateral case reduced ⁹⁹Tc^m-pertechnetate was found to accumulate at the thrombus site after homolateral injection. Further study is necessary to establish this result statistically, but the result points towards that as a radiochemical impurity, reduced ⁹⁹Tc^m-pertechnetate might be responsible for an increased accumulation of radioactivity found at the thrombus site after injections of radiopharmaceuticals with a low labelling yield.

In conclusion, all groups of radiopharmaceuticals investigated showed only slightly more activity in the thrombus than in blood, both *in vivo* and *in vitro*, after contralateral injections. Thus we could not find any difference between radiopharmaceuticals supposed to be actively involved in the thrombotic processes and radiopharmaceuticals without any known relation to these processes. Furthermore,

we did not find any relationship between the size of the radiopharmaceutical and the thrombus uptake. Since the control substances showed similar results to the other radiopharmaceuticals when injected contralaterally, a common mechanism other than a specific thrombus uptake seems to be the reason for the increased measured count rate of radioactivity in the diseased leg of patients with suspect DVT. A more diffuse accumulation in the diseased leg has also been reported in clinical studies regarding $^{99}\text{Tc}^{\text{m}}$ -plasmin, $^{99}\text{Tc}^{\text{m}}$ -erythrocytes, $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid and $^{99}\text{Tc}^{\text{m}}$ -pertechnetate [12, 31, 37, 38], inferring the question whether radiopharmaceuticals in this situation merely illustrate blood pool changes.

After homolateral injection, which mimics γ -phlebography, radiocolloids and reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate showed a high *T/B* ratio while the other radiopharmaceuticals including $^{99}\text{Tc}^{\text{m}}$ -pertechnetate showed a low *T/B* ratio. Thus, radiopharmaceuticals supposed to be actively involved in coagulation and fibrinolysis were found to accumulate less at the thrombus site than radiolabelled colloids.

Furthermore, since reduced $^{99}\text{Tc}^{\text{m}}$ -pertechnetate showed a high thrombus uptake *in vivo* and *in vitro*, the role of radiochemical impurities as a factor determining the usefulness of radiopharmaceuticals for diagnosis of DVT in the clinic cannot be ruled out.

Other impurities such as colloids might also be responsible for an increased uptake [20, 39, 40].

This study also emphasizes that in order to evaluate any radiopharmaceutical for DVT diagnosis thorough quality control is necessary for the correct interpretation of the experimental findings. The question of the mechanism of thrombus entrapment still remains unanswered and further studies should be encouraged.

Acknowledgements

This study has been supported by grants from the John and Augusta Perssons Foundation for Scientific Medical Research, the Swedish Medical Council grant B83-17X-6530-01, Thorsten and Elsa Segerfalks Foundation for Medical Research, Malmöhus Läns Landsting and the Swedish Association against Heart and Chest Diseases.

References

1. Havig Ö. Deep vein thrombosis and pulmonary embolism. *Acta Chir Scand* 1977; Suppl 478.
2. Prentice AG, Lowe GDO, Forbes CD. Diagnosis and treatment of venous thromboembolism by consultants in Scotland. *Br Med J* 1982; **285**: 630–2.
3. Haeger K. Problems of acute deep venous thrombosis. *Angiology* 1969; **20**: 219–23.
4. Bettman MA, Paulin S. Leg phlebography: The incidence, nature and modification of undesirable side effects. *Radiology* 1977; **122**: 101–4.

5. Albrechtsson U, Olsson CG. Thrombotic side effects of lower limb phlebography. *Lancet* 1976; **1**: 723–4.
6. Hull R, Hirsh J, Sackett DL *et al.* Cost effectiveness of clinical diagnosis. Venography and non-invasive testing in patients with symptomatic deep-vein thrombosis. *New Engl J Med* 1981; **304**: 1561–7.
7. Edenbrandt CM, Anderson L, Larsson I *et al.* Diagnosis of deep venous thrombosis by $^{99}\text{Tc}^{\text{m}}$ -human serum albumin microcolloid. *Eur J Nucl Med* 1983; **8**: 332–4.
8. Nicolaides AN, Olsson CG. Applications of isotope technology to the clinical study of arterial and venous disease. The $^{99}\text{Tc}^{\text{m}}$ plasmin. In: Bernstein EF, ed. Noninvasive techniques in vascular disease. London: Mosby, 1982; 159–60.
9. Harwig SSL, Harwig JF, Coleman RE *et al.* *In vivo* behaviour of $^{99}\text{Tc}^{\text{m}}$ -fibrinogen and its potential as a thrombus-imaging agent. *J Nucl Med* 1976; **17**: 40–6.
10. Nilsson JLG, Stenberg P, Ljunggren C *et al.* Fibrin-stabilizing factor inhibitors. *Ann New York Acad Sci* 1972; **202**: 286–96.
11. Webber MM. Labelled albumin aggregates for detection of clots. *Sem Nucl Med* 1977; **3**: 253–61.
12. Beswick W, Chmiel R, Booth R *et al.* Detection of deep venous thrombosis by scanning of $^{99\text{m}}$ -technetium-labelled red-cell venous pool. *Br Med J* 1979; **1**: 82–4.
13. Kempi V, van der Linden W. Diagnosis of deep vein thrombosis with *in vivo* $^{99\text{m}}$ Tc-labelled red blood cells. *Eur J Nucl Med* 1981; **6**: 5–9.
14. Fenech A, Hussey JK, Smith FW *et al.* Diagnosis of deep vein thrombosis using autologous indium-111-labelled platelets. *Br Med J* 1981; **282**: 1020–2.
15. Knight LC, Primeau JL, Siegel BA *et al.* Comparison of ^{111}In -labelled platelets and iodinated fibrinogen for the detection of deep vein thrombosis. *J Nucl Med* 1978; **19**: 391–4.
16. English D, Andersen BR. Organ distribution of canine leukocytes labelled with $^{99\text{m}}$ Tc-sulfur colloid. *J Nucl Med* 1977; **18**: 289–95.
17. Persson BRR, Naversten Y. Technetium-99m-sulfide colloid preparation for scintigraphy of the reticuloendothelial system. *Acta Radiol Ther Biol* 1970; **9**: 567–76.
18. Persson B, Olsson CG, Darte L *et al.* Preparation and testing of $^{99}\text{Tc}^{\text{m}}$ -labelled plasmin for thrombus detection. *Prog Radiopharm* 1981; **2**: 147–56.
19. Olsson PI. Radiolabelling of blood cells with 51-Cr, 99-Tc-m and 111-In. Applications to diagnosis of abscesses, pulmonary platelet trapping and spleen function in animals. Lund, Sweden: University of Lund, internal report LUNFD6, NFRA3025, 1981; 1–22.
20. Bergqvist L, Strand SE, Persson RBR. Particle sizing and biokinetics of interstitial lymphoscintigraphic agents. *Sem Nucl Med* 1983; **13**: 9–19.
21. Persson RBR, Darte L. Gel chromatography column scanning for the analysis of $^{99}\text{Tc}^{\text{m}}$ -labelled compounds. *J Chromatogr* 1974; **101**: 315–26.
22. Pettit WA, Deland FH, Pepper GH *et al.* Characterization of tin-technetium colloid in technetium-labelled albumin preparations. *J Nucl Med* 1978; **19**: 387–92.
23. Cobble JW. Empirical considerations of entropy. I. The entropies of the oxyanions and related species. *J Chem Phys* 1953; **9**: 1443–6.
24. Boyd GE. Technetium and promethium. *J Chem Ed* 1959; **1**: 3–14.
25. Phillips DS. Basic statistics for health science students. San Francisco: WH Freeman, 1978.
26. Cohen Y. Chemical and radiochemical purity of radioactive pharmaceuticals related to their biological behavior. In: Andrews GA, Kniseley R, Wagner HN, eds. Radioactive pharmaceuticals, vol. 6, 1966; 69–91 (AEC Symposium, Oak Ridge).
27. Hobbs JT. External measurement of fibrinogen uptake in experimental venous thrombosis and other local pathological states. *Br J Exp Path* 1962; **43**: 48–58.

28. Webber MM, Bennet LR, Cragin M *et al.* Thrombophlebitis – demonstration by scintiscanning. *Radiology* 1969; **92**: 620–3.
29. Vieras F, Barron EL, Parker GA *et al.* Experimental evaluation of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid as a potential imaging agent in thromboembolic disease: Concise communication. *J Nucl Med* 1980; **21**: 723–8.
30. Bardfeld PA, Rand J, Goldsmith SJ. The use of technetium $^{99\text{m}}$ sulfur colloid as a marker for experimental venous thrombosis: Concise communication. *J Nucl Med* 1981; **22**: 598–600.
31. Bardfeld PA, Harrison DA, Wasserstein G *et al.* Detection of deep venous thrombosis with $^{99\text{m}}$ Tc-sulphur colloid. *Radiology* 1983; **146**: 185–9.
32. Ryo YU, Colombetti LG, Polin SG *et al.* Radionuclide venography: Significance of delayed washout, visualization of the saphenous system. *J Nucl Med* 1976; **17**: 590–5.
33. Olsson CG, Albrechtsson U, Darte L *et al.* Tc- $^{99\text{m}}$ plasmin for rapid detection of deep vein thrombosis. *Nucl Med Commun* 1982; **3**: 41–52.
34. Edenbrandt CM, Nilsson J, Ohlin P. Diagnosis of deep venous thrombosis by phlebography and $^{99}\text{Tc}^{\text{m}}$ -plasmin. *Acta Med Scand* 1982; **211**: 59–64.
35. Overgaard K. Thromboscintigraphy with $^{99}\text{Tc}^{\text{m}}$ -plasmin: a new diagnostic method in deep venous thrombosis of the leg. *Nucl Med Commun* 1984; **5**: 101–7.
36. Edenbrandt CM, Dahlström JA, Nilsson J *et al.* Comparison between $^{99}\text{Tc}^{\text{m}}$ -porcine plasmin and $^{99}\text{Tc}^{\text{m}}$ -labelled erythrocytes in diagnosis of deep vein thrombosis. *Clin Physiol* 1984; **4**: 243–52.
37. Kempf V, von Scheele C. Diagnosis of deep-vein thrombosis with sodium pertechnetate. *J Nucl Med* 1976; **17**: 109–9.
38. Luckers AEG, Luth WJ, Teule GJJ *et al.* Diagnosis of deep venous thrombosis with Tc- $^{99\text{m}}$ -plasmin using the gamma camera. Sixth congress of the European Nuclear Medicine Society, Brussels, Belgium, 1983, abstract.
39. Rhodes BA, Croft BY. Basis of radiopharmacy. St Louis: Mosby, 1978.
40. Syhre R, Spies H, Johannsen B. Organverteilung von $^{99\text{m}}$ Tc(Sn)-hydroxidkolloid bei der Ratte in Abhängigkeit vom preparations-pH. *Radiobiol Radiother* 1976; **17**: 747–50.
41. Gangnon WF. Review of medical physiology. Los Altos: Lange Medical Publications, 1979.
42. Ennis JT, Elmes J. Radionuclide venography in the diagnosis of deep venous thrombosis. *Radiology* 1977; **125**: 441–9.
43. Novo Industri A/S, Copenhagen. Lysofibrin, data sheet, 1973.
44. Nilsson JLG, Lunden R, Lorand L *et al.* Fibrin-stabilizing factor inhibitors. *Ann New York Acad Sci* 1972; **202**: 286–96.
45. Powers WJ, Siegel BA. Thrombos imaging with indium-III platelets. *Sem Thrombosis Haemostasis* 1983; **9**: 115–31.

Diagnosis of Deep Venous Thrombosis by ^{99m}Tc -Human Serum Albumin Microcolloid

C.M. Edenbrandt¹, L. Andersson², I. Larsson², J. Nilsson³, P. Ohlin⁴, S.E. Strand², and F. Ståhlberg²

¹ Department of Medicine, Central Hospital, Helsingborg, Sweden

² Department of Radiation Physics, University Hospital of Lund, Sweden

³ Department of Diagnostic Radiology and

⁴ Department of Clinical Physiology, Central Hospital, Helsingborg, Sweden

Abstract. ^{99m}Tc -human serum albumin microcolloid (HSAC) was evaluated for diagnosis of deep venous thrombosis (DVT) in the legs of 38 consecutive patients. Five and thirty minutes after IV injection, the ^{99m}Tc -HSAC activity in both legs was measured by external counting using a collimated NaI (TI)-detector. The relative predominance of ^{99m}Tc -HSAC activity in the diseased leg was calculated. Phlebography was performed as a control after the ^{99m}Tc -HSAC test. In our hands the non-invasive ^{99m}Tc -HSAC test appeared to be rapid, easy to perform and convenient to the patient. The test showed a high sensitivity (11/13) and a low specificity (10/20) compared with phlebography. No adverse reactions were found. The results seem promising and further studies are in progress to establish the value of the ^{99m}Tc -HSAC test in screening for DVT.

Materials and Methods

Patients

Thirty-eight patients, 24 females and 14 males, with suspected DVT were studied. Twenty-nine patients were consecutively referred from the medical emergency ward, two were referred from the Department of Geriatrics, one patient from each of the Departments of Gynecology, Infectious diseases, Orthopaedics, and Psychiatry. Three patients were referred from general practitioners. Of these thirty-eight patients five were excluded since phlebography was not performed. In all other patients the ^{99m}Tc -HSAC test and phlebography were performed within 24 h. The ^{99m}Tc -HSAC test always preceded the phlebography. All phlebograms were re-evaluated by an experienced radiologist (J.N.) who was unaware of the outcome of the ^{99m}Tc -HSAC test. The results of this re-evaluation were considered to be the definitive outcome of the phlebographies.

^{99m}Tc -HSAC

A freeze-dried, sterile, pyrogen-free mixture of 2.5 mg human serum albumin microcolloid and 0.34 mg stannous chloride was provided in each kit (ALBU-RES, Solco Nuclear Ltd., Switzerland). The kits were stored at 4°C. The labelling procedure took place every morning by adding 5 ml, about 400 MBq, sterile, pyrogen-free ^{99m}Tc -pertechnetate solution to the kit. The vial was gently swirled and the solution incubated at room temperature for at least 5 min. The amount of unbound ^{99m}Tc -pertechnetate was estimated in two kits by gamma camera imaging of a gel chromatography column (Sephadex G-25 Medium, Pharmacia, Sweden) (Persson and Darte 1974; Darte and Persson 1980) and found to be less than 2% (manufacturers value 0%–5%). The mean particle size was previously assumed to be about 500 nm which corresponds to about 10^{10} particles per ml kit solution (Bergqvist et al. 1983).

The ^{99m}Tc -HSAC test

The ^{99m}Tc -HSAC test was performed in a similar way as previously described for the ^{99m}Tc -plasmin test by Edenbrandt et al. (1982). Twelve points were marked on each leg. One foot point (on the medial malleolus), five points on the lower leg (equally distributed between the medial malleolus and the medial fissure of the knee), one knee point and five points on the upper leg (equally distributed between the medial fissure of the knee and the inguinal ligament). The most proximal point on the lower leg and

Introduction

The well-known unreliability of clinical diagnosis of deep venous thrombosis (DVT) makes laboratory tests necessary (Haeger 1969). The most common method today is phlebography which, however, has disadvantages. It is inconvenient and painful for the patient, it may cause thrombosis, it is expensive and thus less suitable as a screening test (Albrechtsson and Olsson 1976; Bettman and Paulin 1977). In the search for a non-invasive, rapid, inexpensive and convenient screening method, an appreciable number of radiopharmaceuticals have been proposed during the last decade (Flanc et al. 1968; Yao et al. 1973; Kempf et al. 1974, 1976; Webber 1977; Beswick et al. 1979; Fenech et al. 1981; Utne et al. 1981; Olsson et al. 1982). Only a few have been evaluated both in an experimental and a clinical setting and far fewer have come into clinical practice.

Recent investigations regarding characterization of colloidal radiopharmaceuticals (Bergqvist et al. 1983) initiated a systematic testing of different colloids for diagnosis of DVT in a rabbit model (Ståhlberg et al. 1982). These experiments pointed to ^{99m}Tc -human serum albumin microcolloid (HSAC) and ^{99m}Tc -sulphur colloid as being suitable for the diagnosis of DVT. Two experimental studies have recently called attention to the value of ^{99m}Tc -sulphur colloid in the diagnosis of DVT (Vieras et al. 1980; Bardfeld et al. 1981). The present study shows the preliminary results of a clinical study in progress, evaluating ^{99m}Tc -HSAC for the diagnosis of DVT.

Offprint requests to: CM Edenbrandt, Department of Medicine, Central Hospital, S-251 87 Helsingborg, Sweden

the knee point were measured from behind. During the measurements, the patient was in a supine position with the legs elevated 45° to minimize blood pooling. The leg points were always measured starting with the right foot point and subsequently alternating between the right and the left leg, in proximal direction.

About 40 MBq (0.35–0.90 ml) ^{99m}Tc -HSA microcolloid was injected IV into an antecubital vein. The count rate at each leg point was measured using a thrombograph (NOVO A/S, Denmark). The apparatus consisted of a collimated NaI (Tl)-detector connected to a scaler-timer, a computer (ABC 80, Scandia-Metric, Sweden) and a printer. Before measuring the leg points, the time for 10^4 pulses over the forehead was determined and used when measuring each leg point. The first measurement of all leg points was started 5 min after the injection and the second measurement was performed 30 min after the injection. If the test was negative at both measurements, it was considered negative. If the test was positive at 5 and/or 30 min after the injection, it was considered positive. The criteria for a positive and a negative test were adopted from earlier experiments with the ^{99m}Tc -plasmin test (Olsson et al. 1982). The count rate recorded during the preset time at each leg point was tabulated. From the count rate at corresponding points of the right and left leg, the relative predominance (R) of the ^{99m}Tc -HSAC uptake was calculated according to the following formula:

$$R = \left[\frac{\dot{N}(\text{Dx})}{\dot{N}(\text{Dx}) + \dot{N}(\text{Sin})} \times 100 \right] - 50$$

$\dot{N}(\text{Dx})$ = count rate at the right leg point,

$\dot{N}(\text{Sin})$ = count rate at the left leg point.

This formula expresses how much the predominant leg differs from the arithmetical mean value for corresponding leg points. The test was judged positive when three adjacent points of the suspected leg showed a $|R| \geq 3$.

Phlebography

Contrast medium (Conray Meglumine, Astra-Meditec, Sweden) was injected into a foot vein and into the femoral vein in the groin (Gullmo 1955; Edenbrandt et al. 1982).

Radiation dosimetry

The effective dose equivalent for the ^{99m}Tc -HSAC-test was calculated to be about 0.5 mSv (Table 1).

Results

A total of 33 patients, 20 females and 13 males, with a mean age of 58.7 years (range 21–91) were studied. The results of the comparison between the ^{99m}Tc -HSAC test and the definitive outcome of the phlebograms are shown in Table 2. The ^{99m}Tc -HSAC test showed a high sensitivity (11/13) and a low specificity (10/20) compared with phlebography. According to the phlebograms, 13 of 33 (39%) patients had DVT. The localisation and extent of the thrombi according to both the ^{99m}Tc -HSAC test and phlebography appeared similar (Table 3).

One patient with a small thrombus corresponding to only one leg point just above the ankle and one patient with a non-occluding thrombus, 15 cm long, localised in

Table 1. The calculated effective dose equivalent of different laboratory tests for diagnosis of DVT

Laboratory test/ Radiopharmaceutical	Injected activity (MBq)	Effective dose equivalent (mSv)
^{99m}Tc -plasmin	20	0.3
^{125}I -fibrinogen	4	0.6
^{99m}Tc -HSAC	40	0.5
Phlebography	—	1.5

Table 2. Comparison between ^{99m}Tc -HSAC test and phlebography for diagnosis of DVT in 33 patients

		Phlebography	
		Positive	Negative
^{99m}Tc -HSAC test	Positive	11	10
	Negative	2	10

Table 3. Comparison between most distal leg point and most proximal leg point with positive ^{99m}Tc -HSAC test (A) and the distal and proximal end of the thrombus as judged from phlebography referred to corresponding leg points (B) in each of 13 patients with DVT

Pa- tient	Distal leg point			Proximal leg point		
	^{99m}Tc - HSAC test (A)	Phlebo- graphy (B)	Differ- ence (A–B)	^{99m}Tc - HSAC test (A)	Phlebo- graphy (B)	Differ- ence (A–B)
1	1	1	0	8	6	+2
2	—	9	—	—	12	—
3	3	1	+2	8	11	–3
4	1	1	0	6	6	0
5	3	1	+2	5	5	0
6	1	3	–2	3	6	–3
7	1	1	0	8	10	–2
8	—	2	—	—	2	—
9	3	1	+2	5	12	–7
10	1	1	0	11	12	–1
11	1	1	0	5	4	+1
12	3	1	+2	12	12	0
13	1	1	0	12	12	0
Mean difference			+0.5	–1.2		

False negative ^{99m}Tc -HSAC test (—)

the middle portion of the femoral vein had negative ^{99m}Tc -HSAC-tests.

Ten patients had false positive HSAC-tests. Most probable diagnoses in these patients were trauma (3), superficial thrombophlebitis (2), neurological disorders (2), erysipelas, congestive heart failure, and unknown cause. Only one of these patients had varicose vessels but he also had superficial thrombophlebitis.

No adverse reaction to the albumin microcolloid was observed during the study.

Discussion

The present results of the ^{99m}Tc -HSAC test are in accordance with earlier findings regarding the ^{99m}Tc -plasmin test (Olsson et al. 1982; Edenbrandt et al. 1982; Adolfsen et al. 1982) and the ^{125}I -fibrinogen uptake test (Olsson 1980) in consecutive patients from a medical emergency ward. Therefore, the utility of the ^{99m}Tc -HSAC test seems to be equivalent to that of the ^{99m}Tc -plasmin test and the ^{125}I -fibrinogen uptake test. Furthermore, the ^{99m}Tc -HSAC-test is superior to the ^{99m}Tc -plasmin test and the ^{125}I -fibrinogen uptake test since it is less expensive and less time-consuming than these tests. The specificity of the ^{99m}Tc -HSAC test could probably be improved without affecting the sensitivity by choosing a more optimal time after injection for measuring the leg points, as has already been shown for the ^{99m}Tc -plasmin test (Edenbrandt and Brudin to be published).

The exact mechanisms for accumulation of radioactivity in the diseased leg have not yet been elucidated for any radiopharmaceutical involved in diagnosis of DVT. Different hypotheses have been proposed from binding to specific sites on the clot surface (Tengborn and Hedner 1981) or involvement in the thrombotic process (Hobbs and Davies 1960) via electrostatic forces, adhesion, trapping by entanglement in microfibrils extending outward from the clot (Webber 1977), to simply reflecting haemodynamic changes secondary to bloodflow obstruction by the clot (Ryo et al. 1976). The similar clinical results obtained with quite different radiopharmaceuticals, emphasised also in this study, implies the question of a common main mechanism of accumulation.

Within the scope of colloids, particle size, number of particles, surface charge of the particles, the agent with which the colloid is stabilised and the injection site are factors with known impact upon the biokinetics of the colloid. Consequently, different colloids, as well as other radiopharmaceuticals, have been tested in an animal model for in vivo diagnosis of DVT (Ståhlberg et al. 1982). The experimental results pointed to ^{99m}Tc -HSAC being suitable for diagnosis of DVT. The present clinical results confirm the value of the animal model to evaluate potential substances for diagnosis of DVT.

Conclusion

The ^{99m}Tc -HSAC test has been found to be non-invasive, rapid, easy to perform, inexpensive, convenient to the patient and the results seem promising when screening for DVT in an emergency ward. Furthermore, the usefulness of a colloid in diagnosing DVT could promote the use of a screening test in clinical practice and advance the knowledge about the mechanisms of action of non-invasive tests in diagnosing DVT.

Acknowledgement. This study was supported by grants from John and Augusta Persson Foundation for Scientific Medical Research, Thorsten och Elsa Segerfalks Foundation for Medical Research, Malmöhus Läns Landsting and the Swedish Council for Medical Research B83-17x-6539-01.

References

- Adolfsen L, Nordenfelt I, Olsson H, Torstensson I (1982) Diagnosis of deep vein thrombosis with ^{99m}Tc -plasmin. *Acta Med Scand* 211:365-368
- Albrechtsson U, Olsson CG (1976) Thrombotic side-effects of lower limb phlebography. *Lancet* 1:723-724

- Bardfield PA, Rand J, Goldsmith SJ (1981) The use of technetium-99m sulfur colloid as a marker for experimental venous thrombosis: Concise communication. *J Nucl Med* 22:598-600
- Bergqvist L, Strand S-E, Persson RBR (1983) Particle sizing and biokinetics of interstitial lymphoscintigraphic agents. *Semin Nucl Med* 13:9-19
- Beswick W, Chmiel R, Booth R, Vellar I, Gilford E, Chesterman CN (1979) Detection of deep venous thrombosis by scanning of ^{99m}Tc technetium-labelled red-cell venous pool. *Br Med J* 1:82-94
- Bettman MA, Paulin S (1977) Leg phlebography: The incidence, nature and modification of undesirable side effects. *Radiology* 122:101-104
- Darte L, Persson RBR (1980) Quality control of ^{99m}Tc -radiopharmaceuticals. Evaluation of GCS minicolumns in routine clinical work with scintillation cameras. *Eur J Nucl Med* 5:521-527
- Edenbrandt CM, Nilsson J, Ohlin P (1982) Diagnosis of deep venous thrombosis by phlebography and ^{99m}Tc -plasmin. *Acta Med Scand* 211:59-64
- Fenech A, Hussey JK, Smith FW, Dendy PP, Bennett B, Douglas AS (1981) Diagnosis of deep vein thrombosis using autologous indium-III-labelled platelets. *Br Med J* 282:1020-1022
- Flanc C, Kakkar VV, Clarke MB (1968) The detection of venous thrombosis of the legs using ^{125}I -labelled fibrinogen. *Br J Surg* 55:742-747
- Gullmo Å (1955) On the technique of phlebography of the lower limb. *Acta Radiol* 46:603-620
- Haeger K (1969) Problems of acute deep venous thrombosis. I. The interpretation of signs and symptoms. *Angiology* 20:219-223
- Hobbs JT, Davies JWL (1960) Detection of venous thrombosis with ^{131}I -labelled fibrinogen in the rabbit. *Lancet* II:134-135
- Kempi V, van der Linden W, von Scheele C (1974) Diagnosis of deep vein thrombosis with ^{99m}Tc -streptokinase: A clinical comparison with phlebography. *Br Med J* 4:748-749
- Kempi V, van der Linden W, von Scheele C (1976) Uptake of ^{99m}Tc -tripolyphosphate in thrombotic leg. *Nuklearmedizin* 1:14-17
- Olsson CG, Albrechtsson U (1980) A modified ^{125}I -fibrinogen technique in suspected deep vein thrombosis. *Acta Med Scand* 207:461-467
- Olsson CG, Albrechtsson U, Darte L, Persson RBR (1982) Tc-^{99m} plasmin for rapid detection of deep vein thrombosis. *Nucl Med Commun* 3:41-52
- Persson RBR and Darte L (1974) Gel chromatography column scanning for the analysis of ^{99m}Tc -labelled compounds. *J Chromatogr* 101:315-326
- Ryo UY, Colombetti LG, Polin SG, Pinsky SM (1976) Radionuclide venography: Significance of delayed washout; Visualization of the saphenous system. *J Nucl Med* 17:590-595
- Ståhlberg F, Strand S-E, Persson RBR (1982) Quantitative animal model for in vivo comparison between radiopharmaceuticals for diagnosis of thrombosis. *Proc Third World Congress Nucl Med Biol* 1664-1667
- Tengborn L, Hedner U (1981) Demonstration of ^{99m}Tc -labelled plasmin and α_2 -antiplasmin on the surface of ex vivo thrombi. In: Davidson JF (ed) *Progress in fibrinolysis*, Volume V, Churchill Livingstone, Edinburgh, pp 324-328
- Utne HE, Pors Nielsen S, Klemp P, Nielsen HV (1981) A gamma camera method for the evaluation of deep-vein thrombosis in the leg. Application of ^{99m}Tc -labelled heparin. *Eur J Nucl Med* 6:237-240
- Webber MM (1977) Labeled albumin aggregates for detection of clots. *Semin Nucl Med* 7:253-261
- Vieras F, Barron EL, Parker GA, Grissom MP (1980) Experimental evaluation of Tc-^{99m} sulfur colloid as a potential imaging agent in thromboembolic disease: Concise communication. *J Nucl Med* 21:723-728
- Yao JST, Henkin RE, Conn J, Quinn JL, Bergan JJ (1973) Combined isotope venography and lung scanning. *Arch Surg* 107:146-151

Received December 28, 1982 / March 12, 1983

